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# MORPHOLOGIC ALTERATIONS OF THE NEURON DUE TO TUMOR INVASION

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What changes does the nervous parenchyma undergo under the impact of invasion by neoplastic tissue? This question is interesting from several points of view. It is of theoretic interest from the point of view of the pathology of the neuron and from that of the biologic behavior of the tumors which invade nervous tissue. It is of clinical interest because general experience shows that the localizing syndromes pointed out by classic neurology on the basis of vascular lesions or mechanical injuries are often largely modified by tumors occurring in the same area. This discrepancy has been pointed out particularly with respect to the extrapyramidal ganglions in 25 cases from Cushing's department (Ody [1]) and in connection with tumors of the thalamus (Smyth and Stern [2]). Scherer [3] studied the changes which the nervous parenchyma undergoes under the influence of pressure from an adjoining meningioma. In the present investigation an attempt was made to study the effect of glioma on the nerve elements.

## MATERIAL AND METHODS

Only postmortem material was studied. It was derived from 15 cases of astrocytoma (piloid, gemistocytic, diffuse), 11 cases of glioblastoma multiforme, 3 cases of astroblastoma, 3 cases of spongioblastoma unipolare, and 4 cases of medulloblastoma—altogether 36 tumors.

The following stains were applied: hematoxylin–Van Gieson (general survey); Nissl (ganglion cells); Mallory's phosphotungstic acid hematoxylin (neuroglia fibers); Bielschowsky (axis cylinders and neurofibrils); Bodian (nerve fibers and nerve endings); Spielmeyer (myelin sheaths); sudan IV (fat); Marchi (degenerated nerve fibers).

## RESULTS

Instead of describing single cases we may summarize the observations as follows:

*Nerve Cells.*—The nerve cells are well preserved in an outer zone of the glioma which varies in depth. They become rarer toward the "interior" part of the neoplasm, but even there one still finds in every type occasional nerve cells which are conspicuously well preserved. In the majority of the cases no transitional stage can be seen between complete preservation and complete disappearance. Where such a change can be seen at all it is of the variety of "simple shrinkage" (Spielmeyer [4]), i. e., a diminution of volume with closer packing of the Nissl bodies and fair preservation of the nucleus (fig. 1). This zone of transition is small in every case and includes only a comparatively small number of cells. The most striking phenomenon is a negative one, i. e., the complete absence of degenerative changes, familiar to all from circulatory, infective, toxic and traumatic conditions, such as the "severe" cell change, the appearance of "homogenization" and the "ischemic" cell change. All these degenerative changes, characterized by various stages and degrees of disintegration of the nucleus, chromatolysis and "liquefaction" are absent.

This is especially striking in certain areas where otherwise the cells show high vulnerability, e. g., the Purkinje cells. These cells, which are known to react even to minor damage with "homogenization" of protoplasm and nucleus, show a completely normal appearance or simple shrinkage when completely engulfed by the tumor cells of medulloblastoma. Even when they are markedly displaced, "carried away" as it were by the growth, they do not show any other changes.

Another instructive example is the nerve cells of the substantia nigra. Because of the melanin, these cells were always particularly valuable for the study of disintegration of ganglion cells in general (Klarfeld [5]; Spatz [6]; Stern [7]). The pig-

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1. Ody, F.: Arch. Neurol. & Psychiat. **27**:249, 1932.
2. Smyth, G. E., and Stern, K.: Brain **61**:339, 1938.
3. Scherer, H. J.: Rev. neurol. **66**:307, 1936.
4. Spielmeyer, W.: Histopathologie des Nervensystems, Berlin, Julius Springer, 1922.
5. Klarfeld, B.: Ztschr. f. d. ges. Neurol. u. Psychiat. **77**:80, 1922.
6. Spatz, H.: Encephalitis, in Bumke, O.: Handbuch der Geisteskrankheiten, Berlin, Julius Springer, 1930, vol. 11.
7. Stern, K.: Ztschr. f. d. ges. Neurol. u. Psychiat. **148**:753, 1933.

ment granules enable one to trace parts of the protoplasm throughout all stages of degeneration, neuronophagia and removal to the perivascular space. In a case of piloid astrocytoma of the midbrain, trial sections were taken from various levels. Intact ganglion cells of the substantia nigra were standing out in the depth of the neoplasm, densely encroached on by tumor cells. forms of necrobiotic change occurring in ordinary brain tissue could be observed.

Stains of intracellular fibrils (Bielschowsky) showed no particular changes.

*Axons and Myelin Sheaths.*—Where white matter is involved the myelin stains show the following picture. At the periphery of the tumor the nerve fibers are slightly separated, as it were,

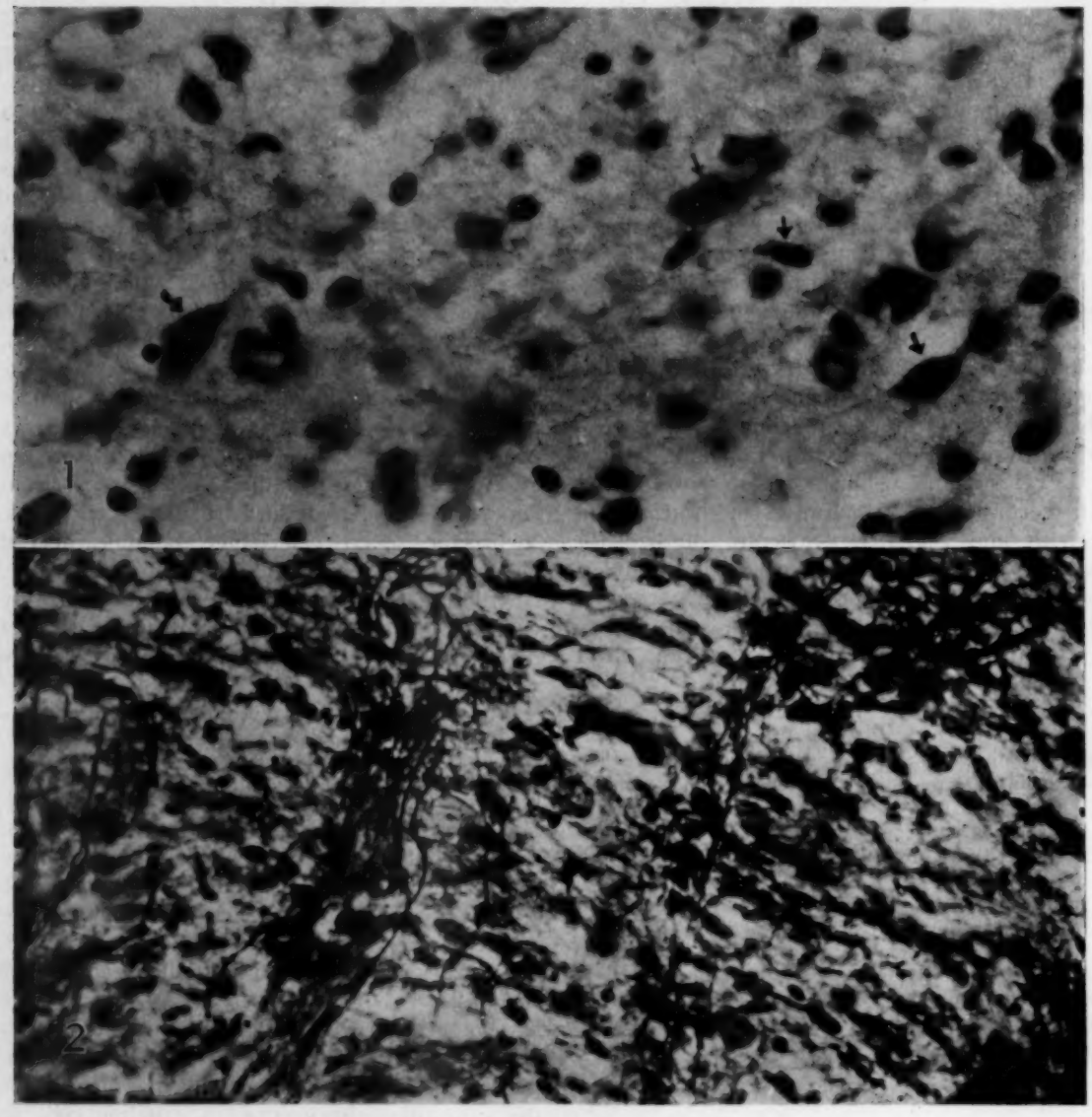

Fig. 1.—Simple shrinkage of nerve cells within piloid astrocytoma (Nissl stain).

Fig. 2.—Glioblastoma multiforme growing in white matter (Spielmeyer stain). In the outer zone of the tumor one can see bundles of beaded and tortuous myelinated fibers. They are separated by the growth, but the parallel course can still be recognized.

There were a few clumps of melanin outside ganglion cells, free in the tumor, but not more than one might expect in a normal midbrain of an adult. The common degenerative changes of nerve cells mentioned were seen only in tumor areas which had undergone necrosis. There all by tumor cells. As one approaches the center of the tumor, there are still bundles of myelin sheaths with maintenance of a more or less parallel arrangement. However, these bundles are separated by masses of tumor cells (fig. 2). The myelin sheath itself shows thinning, swelling

and beading at irregular distances. In still deeper zones of dense growth one sees a picture very characteristic of this beading, with fragments of nerve fibers, which are quite irregularly arranged (fig. 3). The whole picture resembles dry twigs on the ground. Not all of these are real fragments. Some of them seem to be irregularly bending fibers which have been cut at

unable to obtain a positive Marchi reaction. Some glial cells contain occasional droplets of neutral fat, but this is obviously not a result of phagocytosis of products of myelin degeneration.

The axis-cylinders show the same changes as far as the irregular arrangement and beading are concerned.

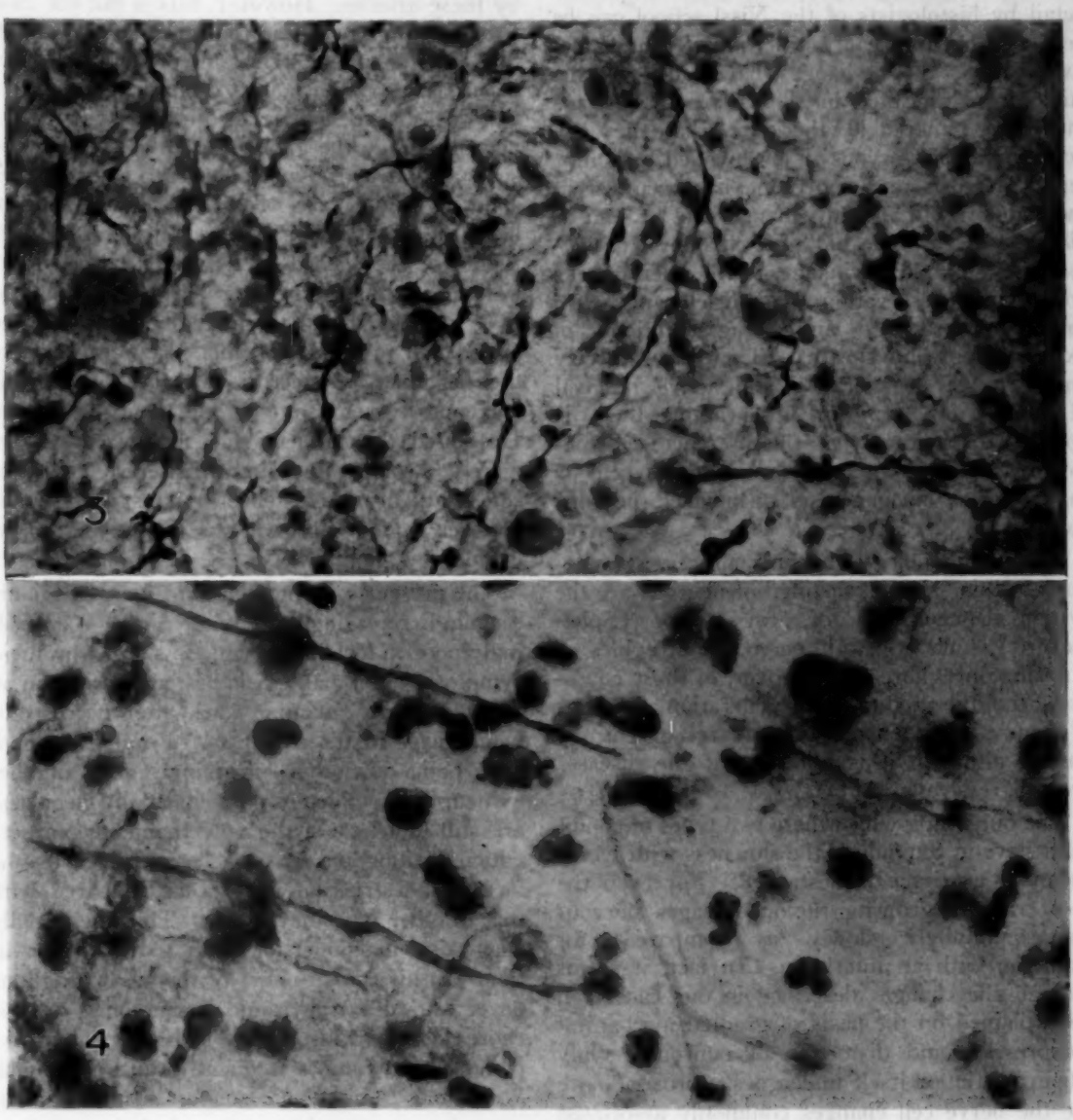


Fig 3.—A deeper zone of the tumor seen in figure 2 (Spielmeyer stain). The beaded, thinned, fragmented myelin fibers present the appearance of scattered "dried twigs."

Fig. 4.—Astrocytoma. The section is from the depth of the tumor. Stained with sudan IV, the thinned myelin fibers present the normal pale pink appearance; there is no neutral fat reaction.

different levels. However, the most important feature seems to be that even here these severely altered nerve fibers still give a myelin reaction and fail to show any fatty degeneration (fig. 4). They stain pale pink except for an occasional bright red droplet with sudan IV. We were also

All types of glioma showed essentially the same types of change. There is, however, a certain difference in the severity of change which coincides with the malignancy of the tumor. Thus, in glioblastoma multiforme the "outer" zone of well preserved neurons is smaller than in

piloid astrocytoma. The diffuse astrocytoma assumes a peculiar position because of the fact that the nervous parenchyma appears morphologically intact throughout.

## COMMENT

It has often been emphasized that the various necrobiotic changes of nerve cells described in detail by histologists of the Nissl school can be produced by different vascular, toxic and infective causes; these changes have little specific etiologic significance in themselves. It seems the more remarkable, therefore, that they are never found in nervous tissue invaded by tumor unless necrosis or hemorrhage has occurred in the tumor itself. If any change can be seen at all, it is of the type of "pure atrophy" (Spielmeyer [4]), simple shrinkage of the nerve cells with fair preservation of nuclei and with thinning, beading and fragmentation of axons and myelin sheaths. There are scarcely any signs of degeneration and active removal of neutral fat. In our observations there is nothing to indicate that the presence of occasional droplets of neutral fat in tumor cells is the result of phagocytosis of invaded nervous tissue.

In neuropathology the only processes analogous to this "simple wasting" of neurons are the system degenerations of gray matter (Spielmeyer [4]), such as certain cerebellar atrophies or Huntington's chorea. Moreover, in those conditions, too, the products of degeneration and signs of removal are conspicuously scarce or entirely absent, although it is known that the diseases are progressive.

The only thing which these two types of conditions have in common is the slowness with which the nervous tissue is destroyed. For even the most rapidly growing tumor damages nervous tissue infinitely slowly as compared, for example, with an infarction. In fact, it seems that all the changes described in our cases can be explained on the basis of gradual mechanical compression and distortion of neurons. Only when the tumor itself undergoes necrosis do we see the nerve cell changes commonly associated with anoxia.

This is especially interesting in connection with Scherer's [8] observations on the cortex underlying meningeal tumors. He described various necrotic changes similar to those seen in circulatory diseases, and he interpreted them as due to interference with the blood supply. He contrasted this with the fact that the cortex overlying a glioma does not undergo the same necrotic changes; he argued that since the supplying blood vessels reach the cortex from the pia mater, they can be interfered with only by meningioma. Apart from the fact that this is not quite correct (the cortex is partly supplied by ascending arteries), it seems reasonable that glioma which invades nervous substance in the vicinity of the supplying arteries and emerging veins would cause necrosis in the tissue supplied by these arteries. However, this is not the case. From the present observations one must conclude that a blood supply sufficient to nourish the neoplasm is also sufficient to nourish the invaded nervous tissue and that the destruction of neurons inside the glioma is due to simple pressure atrophy. What happens to entire hemispheres in the case of hydrocephalus happens here to single cells and their processes when these are encroached on by tumor tissue. MacCallum,[8] in discussing the contrast between the liver in typhoid and the liver densely infiltrated by cells in myeloid leukemia, drew attention to the fact that no necrosis can be seen in the leukemic liver in spite of the huge infiltration. He stated that in leukemia the liver cells are merely "compressed into flattened rows or squeezed out of existence." This corresponds precisely to our observations on nervous tissue invaded by glioma.

The present investigation explains why glioma seemingly replacing brain tissue can be so exceedingly poor in localizing symptoms and why these symptoms often make their first appearance in an "apoplectiform" manner, i. e., after necrosis or hemorrhage in the neoplasm. From a morphologic point of view a surprisingly great number of neurons completely surrounded by neoplasm are still intact. On the other hand, the preservation of function can also be explained by the fact that destruction is slow as compared with that in other types of damage. This is especially suggestive in connection with Ody's [1] observation. In his cases tumors of the basal ganglions never caused extrapyramidal symptoms, such as chorea and parkinsonism. It is a common experience that tumors frequently cause pyramidal symptoms. This would suggest that slow invasion of a system of complicated chains of short neurons leaves sufficient time for "detour" conduction of stimuli whereas in the case of one single long neuron no such possibility exists. However, this is only offered as a speculative explanation.

The present investigation also indicates that nerve elements deep within the tumor do not

8. MacCallum, W. G.: A Textbook of Pathology, Philadelphia, W. B. Saunders Company, 1939.

in case of doubt justify the assumption of a neuroblastic origin of the neoplasm.

SUMMARY

In cases of various types of glioma the effect of the growth on invaded nervous parenchyma was studied. All types of glioma produce in this respect essentially the same picture although this may differ in degree. Completely intact neurons can be seen over a varying depth within the tumor. Frequently there is no transition between complete preservation and complete disappearance of nerve cells. Where there is a change of nerve cells at all it is of the variety of simple atrophy, which is probably due to pressure. There are peculiar changes of axons. All those changes of cells and processes frequently encountered in vascular, toxic and infective conditions are entirely absent unless the neoplasm itself undergoes secondary changes. The foregoing observations have pathologic and clinical significance.

# MORBID ANATOMY OF TYPHUS AS SEEN IN A RECENT GUATEMALAN EPIDEMIC

## OBSERVATIONS ON THE DISEASE

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On April 3, 1944 epidemic typhus broke out in the Asilo de Alienados (hospital for patients with mental diseases), adjacent to the General Hospital, in Guatemala City. Through the cooperation of Drs. Julio Roberto Herrera and Enrique Padilla of the Sanidad Publica of Guatemala, numerous blocks of autopsy tissue fixed in solution of formaldehyde were forwarded to me for study at the Army Medical Museum, Washington, D. C. A total of 10 deaths were investigated by that method. The public health aspects of this outbreak have been described by Herrera.[1] Martinez Duran[2] has described certain of the pathologic aspects. The purpose of this paper is to draw attention to some interesting features of the morbid anatomy as seen in the tissues submitted, particularly those features which have not received heretofore the attention which I believe they deserve.

### MATERIAL AND METHODS

Certain organs and tissues were not represented in the sample tissues available. For example, no blocks of the endocrine glands other than the adrenal glands were submitted. Only occasional blocks of the gastrointestinal tract were resected for study. On the other hand, multiple blocks of the myocardium, the central nervous system, the liver, the spleen and the kidney were submitted from each case.

It is proposed to present the observations on the available tissues by systems and organs. All sections were stained with hematoxylin and eosin. In 1 case organisms consistent with morphologic descriptions of Rickettsia were observed in the testicular tissues after staining the sections overnight in Giemsa solution until the cytoplasm of the swollen endothelial cells lining small blood vessels became deep blue and then decolorizing until the cytoplasm was faintly blue, but retaining the sharp delineation of cell borders. With this method of staining the forms compatible with Rickettsia were deep blue and were seen against a nongranular pale blue endothelial cytoplasm.

Suggestions as to technic were contributed by Dr. Arnold Rich; the technical procedures were carried out by Sergeant I. Wodinski.

This study was made possible through cooperative effort of the Institute of Pathology of the Army Medical Museum, Washington, D. C., the Division of Health and Sanitation, Office of the Coordinator of Inter-American Affairs, Washington, D. C., and the Inter-American Cooperative Public Health Service in Guatemala.

1. Herrera, J. R.: Bol. Ofic. san. panam. **23**:603, 1944.

2. Martinez Duran, C.: Juventud med., 1944, p. 1; Bol. Ofic. san. panam. **23**:791, 1944.

### OBSERVATIONS

*Cardiovascular System.*—Of the numerous blocks of myocardium submitted, most of them from the left ventricular wall, many but not all showed variably severe interstitial myocarditis. In 4 cases only one or a few blocks of heart muscle showed the lesion while others did not. On the basis of such distribution it is assumed that interstitial myocarditis was a common if not universal lesion in this epidemic, but patchy in its distribution in a given heart. Most of the muscle fibers in affected zones were rather widely separated by both cellular infiltrations and edema. The infiltrating cells were predominantly mononuclear forms—monocytes and lymphocytes—with lesser numbers of polymorphonuclear leukocytes and only occasional eosinophils. Occasionally a necrotic muscle fiber or a fiber showing necrosis of a portion of its length could be identified, characterized by swelling of the muscle substance, loss of striations and deep homogeneous hematoxylin staining of the ground substance. In contrast with sections of brain and testicle in which blood vessels showing typical reaction to rickettsial parasitization were numerous and seen with particular ease, those of heart muscle showed relatively few vascular lesions. While serial sections were not made in order to explore thoroughly the relationship of vascular lesions to interstitial myocardial inflammation, multiple step or interval sections were examined in 3 cases without finding any evidence that the two were closely related. It appears reasonable to assume therefore that interstitial myocarditis as seen in this epidemic represents a primary insult to the heart muscle and not necessarily an interstitial cellular response to vascular injury.

Lesions of the smaller vessels could be found in all the tissues examined but were particularly plentiful in blocks of the central nervous system, including the brain stem and the spinal cord. Most commonly, but not universally, small arteries and arterioles were the branches of the arterial tree involved. The lesions varied considerably in degree in different cases and in different blocks from the same case. Simple endothelial swelling, varying from slight to

marked, and the formation of two and even three layers of endothelial cells were the most common lesions. Occasionally a fresh hyaline thrombus could be seen attached to the swollen endothelium, partially or completely blocking the lumen (fig. 7). The degree of endothelial swelling was estimated as a two to five fold increase of cell volume. The regional cellular reaction to the vascular injuries will be described in connection with the various organs and tissues.

There was no opportunity in this survey to examine portions of major veins, arteries or aorta.

In 1 case extensive fresh thrombosis of the endocardial surface was observed with no evidence of concomitant infarction of the muscle fibers. It was assumed that the thrombus was superimposed on direct endocardial injury.

*Central Nervous System.*—In the blocks of the cerebral hemispheres the most common lesion was the vascular response already described. There appeared to be no predilection for any one area or for gray matter as opposed to white matter, except that sections of pons and medulla showed, as a rule, more severe and more numerous lesions than other blocks of the central nervous system. In contrast with the myocardial lesion described, lesions of the central nervous system bore an intimate relation to vascular injuries. While many vascular lesions were surrounded by apparently normal tissue, this was not the rule. Marked vacuolation of nervous tissue regional to involved vessels was common, sometimes extending for as much as the diameter of a low power microscopic field around such vessels. In numerous sections of this series there were several such areas which showed in addition considerable glial mobilization and the presence of compound granular cells. Leukocytes were either scanty or entirely absent in these areas (fig. 1).

Around other vessels showing endothelial hyperplasia and hypertrophy there were collars of glial cells ranging from a single row to a few rows of closely packed cells. Other sections showed fresh perivascular hemorrhages, sometimes extending over more than one low power microscopic field and recognizable grossly.

A striking alteration but by no means a universal one were zones of bland necrosis in which the ground substance showed the shadows of the previous living tissue. No cellular reactions were observed around these areas in some instances, and in others mild glial and astrocytic collars were present. The most extensive zone of necrosis was seen in one section of pons (fig. 2).

Nodules of Frankel, so frequently noted by many others in epidemic typhus, were found in profusion in this series. Their relationship to affected blood vessels was frequently obvious. They were of variable composition, ranging from a scattering of glia cells in a zone of partial necrosis to a dense aggregation of a hundred or more such cells with a well defined center of bland necrosis.

The leptomeninges showed round cell meningitis, apparently patchily distributed. In the meshes of the pia-arachnoid, loosened by edema, there were from 5 to 50 round cells per low power microscopic field, chiefly lymphocytes, plasma cells and large mononuclear phagocytes (fig. 3).

All of the changes described in the foregoing paragraphs, including zones of necrosis, were seen in sections of the spinal cord in the 2 cases in which the cord was submitted. As in the other portions of the central nervous system, vascular reactions, perivascular zones of necrosis, hemorrhages and nodules of Frankel were distributed apparently haphazardly, affecting both gray and white matter. The meningeal reaction was pronounced in one of these 2 cases and mild in the other.

*Adrenal Glands.*—Only 4 pairs of adrenal glands were submitted from this series of cases, and they showed a uniform lesion, varying only in degree in different sections and different cases. The essential features of the lesion were simple necrosis and solution of individual cells or groups of cells, which destroy the continuity of the adrenal gland cords and, when marked, produced lacunas in the substance of the gland. In less extreme examples the normal solid cord became transformed into pseudoglandular foci (figs. 4 and 5). Leukocytic reactions were absent in every part of the adrenal gland examined except the periadrenal adipose tissue, where diffuse and focal lymphoid infiltrations were seen with and without specific vasculitis.

The significance of the lesion of the adrenal gland will not be proved until biochemical studies of adrenal gland function can be made in the living patient. It is interesting to note that the same or a very similar lesion has been redescribed recently by Rich[3] in a variety of septicemic states, including meningococcic septicemia. The lesion may be of considerable importance in the genesis of the severe shocklike state so common clinically in Guatemalan typhus.

*Spleen.*—The hemopoietic system as a whole cannot be discussed in this paper inasmuch as the only representative of the organs and tissues comprising the system were numerous blocks of spleen. In most of the cases either one or more lesions of the following types could be

3. Rich, A. R.: Bull. Johns Hopkins Hosp. **74**:1, 1944.

seen: (*a*) hemorrhagic foci or zones and (*b*) zones of necrosis.

In the hemorrhagic areas there were masses of fresh blood covering red and white pulp indiscriminately, without any signs of organization or of undue leukocytic response. These lesions appeared to be terminal phenomena.

The necrotic areas varied from pallid zones a fraction of a low power microscopic field in diameter to those as large as a low power microscopic field. The framework of the pulp, red or white, usually could be recognized, at least in part, as the background for these zones of anemic necrosis.

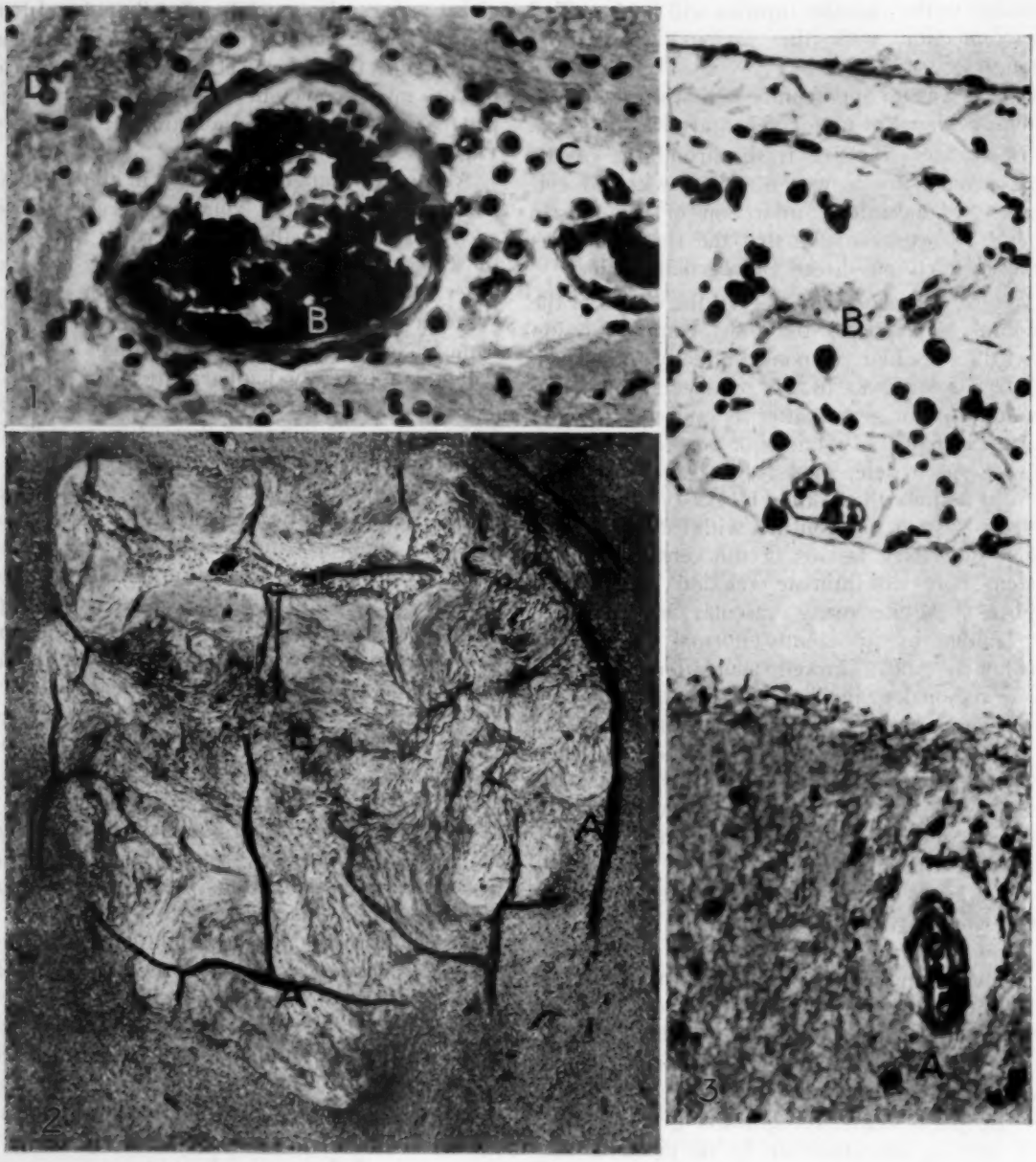


Fig. 1.—Section of cerebral cortex; × 383. Endothelial hypertrophy is seen at *A*. The terminal portion of a thrombus (*B*) partially occludes the lumen. The perivascular tissue shows encephalomalacia (*C*) and contains several compound granular cells (*D*). (Army Medical Museum Negative 80867.)

Fig. 2.—Section of pons; × 43. The heavy lines (*A*) are artefacts due to wrinkling of the section. There is a large zone of ischemic necrosis (*B*), along one border of which is a thrombosed vessel with a thick collar of round cells about it (*C*). Note that there is practically no cellular reaction around this large zone of necrosis. (Army Medical Museum Negative 80866.)

Fig. 3.—Section of cerebral cortex showing the pia-arachnoid; × 383. A vessel showing marked endothelial hypertrophy is seen at *A*. The pia-arachnoidal meshwork is loose and contains numbers of plasma cells, lymphocytes and monocytes (*B*). (Army Medical Museum Negative 80633.)

In neither type of lesion could specific vasculitis be incriminated as the cause, although serial or step sections were not undertaken by way of investigation.

In the absence of lesions of either or both of the aforementioned types, the sections of spleen presented no significant changes other than mild and inconstant hyperplasia of pulp cells.

*Liver.*—Hypoplasia of cells, chiefly central in distribution, was not uncommon, merging occasionally with zones of frank necrosis, without hemorrhage. In other areas focal necroses not unlike those seen in typhoid were present, occasionally in considerable numbers. Specific vascular lesions were observed only occasionally and appeared to be confined to the portal spaces, where small granulomatous foci surrounded such vessels, appearing not unlike the nodules of Frankel in the brain.

*Skin.*—The superficial portions of the corium of exanthematic plaques showed numerous specific vascular lesions similar in all respects to those seen in the brain. Unaffected vessels were usually congested. Lymphangiectasia was com-

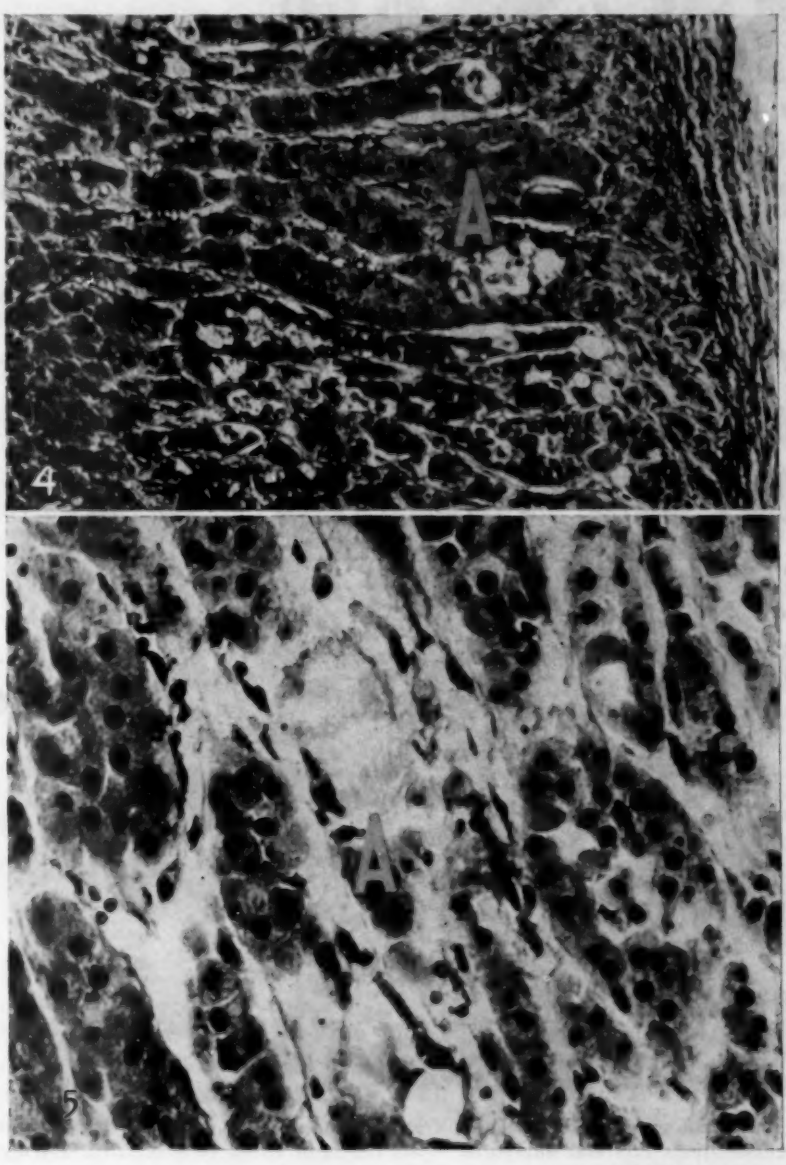


Fig. 4.—Section of the cortex of an adrenal gland; × 120. Necrosis of individual cells has resulted in irregular lacunas (*A*) dispersed haphazardly in the cortical substance of the gland. (Army Medical Museum Negative 80830.)

Fig. 5.—View of a portion of the section shown in figure 4 under higher power; × 400. Note the sudden break in the continuity of the adrenal gland cord (*A*) with solution of a number of cells. Note that the sinusoidal border can be identified in the necrotic area and that remnants of cytoplasm are still visible. (Army Medical Museum Negative 80831.)

mon, even in the vessels lying deep in the corium. Unorganized hemorrhages were seen frequently and tended to be located superficially in the corium. No changes could be seen in the overlying epidermis.

Of special interest was one section presumably from a large macular, perhaps confluent exanthematous plaque. The lesion was characterized not only by the specific vasculitis just described but by widespread hemorrhagic necrosis including the epidermis and extending down to the subcutaneous adipose tissue. The lesion was such that one could predict sloughing of that area in time. It is suggested that a lesion of that type may be of significance in the genesis of typhus ulcers.

*Kidneys.*—Numerous blocks showed one or more examples of the lesions to be described, among which specific vasculitis was inconspicuous. Yellow to brown homogeneous casts were seen, particularly in the distal portions of the convoluted tubules, and occasionally were present in profusion. In the same sections, as well as in those from different cases, other

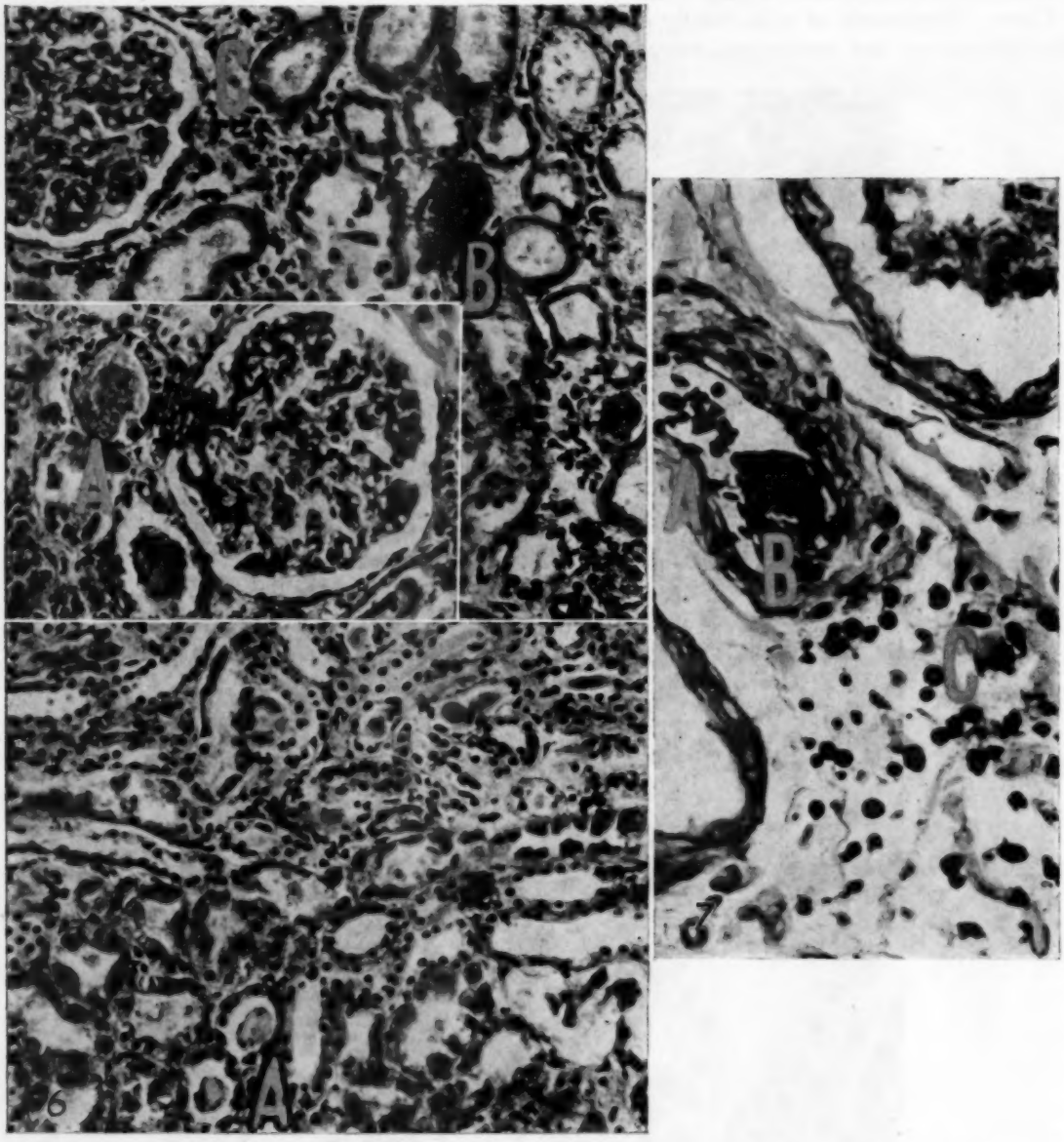


Fig. 6.—Composite photograph of renal lesions in 3 different cases, showing brown-pigmented casts (*A*), red blood cell agglomerations (*B*) and interstitial round cell infiltrations (*C*); × 220.5. (Army Medical Museum Negatives 80925, 80927 and 80926.)

Fig. 7.—Section of testicle showing an interstitial vessel with endothelial hyperplasia (*A*) and thrombus formation (*B*) with regional round cell infiltration (*C*); × 383. (Army Medical Museum Negative 80636.)

tubules had frank agglomerations of red blood cells in similar situations. The pigmented casts were consistent in tinctorial properties with those of hemic origin. Inasmuch as pigmented cast–red blood cell mixtures were also seen, the supposition appears warranted that the lesion represents a hemic or hemoglobinuric nephrosis of undetermined genesis. Simple vascular lesions of the glomerular tuft caused by rickettsial injury were not seen, although occasional hemorrhages in Bowman's capsule were noted. These appeared to be all too slight in degree to account entirely for the tubular lesions. Occasional foci of necrosis of the tubular epithelium adjacent to the casts were present and tended to form minute pseudogranulomatous foci composed of round cells and epithelial cells, spilling over into the interstitial tissues. In addition to the tubular lesions, and in some cases apparently independent of them, there was mild to severe subacute interstitial nephritis characterized by infiltrations of lymphocytes, plasma cells, lesser numbers of monocytes and polymorphonuclear leukocytes, limited neither to the cortex nor to the medulla. There was only minimal and at times no leukocytic infiltration around the pelves or calices, distinguishing the lesion from ascending pyelonephritis (fig. 6).

*Testes.*—In the 6 cases in which testicular tissues were submitted, all sections showed the specific vasculitis characteristic of rickettsial infection either in the tunica albuginea or in the interstitial tissues of the testicle, or in both areas. If one may judge the extent or the severity of the vascular reaction from this form of random sampling of tissue specimens, it appears that the lesion was particularly severe in the testicle and its tunic. The vascular reaction with partial thrombosis of the vessel and regional interstitial round cell infiltration illustrated in figure 7 is typical of this series. Specific tubular lesions and damage to the interstitial tissues were not observed.

*Esophagus.*—Several blocks from the gastrointestinal tracts of 2 patients were the only ones of this organ submitted for examination. Each showed submucosal round cell infiltration, usually in discrete foci and of moderate degree. No ulceration of the mucosa was noted. The significance of the lesion, which again appeared to be independent of specific vasculitis, is obscure.

## COMMENT

The lesions of louse-borne epidemic typhus as seen in 10 fatal cases in this outbreak may be classified in two large groups. One group consists of the specific vascular lesions of rickettsial infection, which needs no further description. The vascular lesions appeared to be most pronounced in the central nervous system and the testes, and they were responsible apparently for a certain degree of destruction or at least inflammation of the regional tissues. The other group of lesions appeared to be independent of, or at least not intimately related to, the vascular lesions and included interstitial myocarditis, meningitis, focal cellular necrosis of the adrenal glands, interstitial nephritis, hemoglobinuric nephrosis and focal necroses of the liver and spleen. Of particular significance were the observations in the spinal cord, which showed lesions identical with those of the cerebral hemispheres, the cerebellum and the brain stem. The lesions of the pons and the medulla were the most severe, both in number and in extent, and included the large areas of bland necrosis seen in figure 2. It was suggested that the severe degree of hemorrhagic necrosis seen in one section of skin could be the pathogenetic precursor of a typhus ulcer.

The clinical state of collapse so remarkable in typhus in Guatemala may be correlated with focal necrosis of the adrenal glands, although one must also consider interstitial myocarditis and the lesions of the central nervous system in the pathogenesis of that state. One can only speculate on the residua or sequelae of such massive necrosis of the central nervous system as was seen in this series in patients that survive the primary illness. Obviously such necrotic areas could heal only by neural scar formation, with clinical sequelae in direct proportion to the exact site and extent of the part of the central nervous system affected.

Hemoglobinuric nephrosis is of some significance because lesions similar to that which characterize this condition are seen in association with severe body burns, massive crushing of muscles ("crush syndrome")[4] and certain shocklike states. I shall not attempt with the inadequate data at hand to speculate on the exact mechanism of this lesion in typhus. The fact nevertheless remains that it exists to a degree comparable to that seen in body burns, for example. Interstitial nephritis of infectious origin is too well known to require further comment. Apparently, epidemic typhus may be added to the list of diseases in which the lesion may be encountered.

## SUMMARY

In addition to vascular and other lesions characteristic of rickettsial infection, this study of 10 cases of typhus revealed foci of necrosis in the central nervous system and in the adrenal glands. In the adrenal glands the necrosis was associated with cytolysis.

4. Bywaters, E. G. L., and Dible, J. H.: J. Path. & Bact. **55**:7, 1943.

# GENETIC ANALYSIS OF THE INDUCTION OF TUMORS BY METHYLCHOLANTHRENE

## VIII. TWO MUTATIONS ARISING IN MICE FOLLOWING INJECTION OF METHYLCHOLANTHRENE

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NEW HAVEN, CONN.

Six genes for susceptibility to methylcholanthrene induction of tumors have been indicated by data which I have recorded.[1] These are, in order of discovery, one on the "agouti" chromosome, one on the "pink eye" chromosome, one on the "piebald" chromosome and three on the X chromosome. The gene on the "pink eye" chromosome and one of the genes on the X chromosome have been demonstrated in tests for linkage following suitable outcrosses. The other four genes are indicated, but the tests for linkage have not been completed. The three genes on the X chromosome probably are allelomorphs. The interpretation that seems to fit the data best for the three sex-linked genes is that a single gene which did not originally give a greater susceptibility to methylcholanthrene induction of tumors in one or the other sex has mutated twice at some time in the past—once in the direction that produces greater susceptibility in the female and again in the opposite direction, producing greater susceptibility in the male. As far as known, these six genes were involved in susceptibility to induction of tumors before the various inbred strains of mice were either established or given injections of methylcholanthrene.

During the past five years an experiment has been performed for the purpose of analyzing the genetic factors obviously involved in the induction of tumors by methylcholanthrene. For this purpose 200 mice (100 females and 100 males) of each of the fifteen inbred strains [2] were used. In addition to these inbred mice several special strains were established through selection following outcrosses. Some of the data have been published.[3]

An analysis of the pertinent data obtained so far discloses that two new germinal mutations have arisen during the course of the experiment in which methylcholanthrene has been injected into hybrid mice. It is the purpose of this paper to present the data that indicate the origin and the transmission of these two mutations.

### MATERIALS AND METHODS

All mice used in this experiment were of the NHO descent and belonged to the same series as those described in previous reports.[3] The NHO mice were originally produced by tandem crosses between mice of the CBA, the JK and the N inbred strains. The idea behind the production of the NHO strain was to obtain a group of mice which would show the greatest possible degree of biologic variability without the interfering incidence of spontaneous tumors. Up to the present time spontaneous tumors have been infrequent, as reported previously. The incidence of all types of spontaneous tumors with the exception of those of the lung is less than 1 per cent. Bronchiogenic carcinoma is the only spontaneous tumor to which the NH (or the derived NHO) stock shows any significant susceptibility (starting at 325 days of life and increasing in numbers up to 30 per cent of all mice by 575 days and to 42 per cent at 675 days of life). In a very small number of these mice mammary tumors, adenocarcinoma of the stomach (in one subline), melanotic tumors, leiomyosarcoma of the uterus and leukemia have been observed. Cystic ovaries and cystic glandular hypertrophy of the uterus are evidences of hormonal dysfunction in mice of this strain—two conditions which increase in frequency with advancing age. Stenosis of the esophagus [4] and glomerulonephritis [5] are of common occurrence in these mice, also.

At 60 days (plus or minus 1 day) all mice were given on the right side of the body a subcutaneous injection of 1 mg. of methylcholanthrene dissolved in 0.1 cc. of sesame oil. Mice were kept either as breeders or as

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1. Strong, L. C.: Rec. Genet. Soc. Am. **12**:56, 1943; unpublished data (records on three stocks C, I and F not complete).

2. Strong, L. C.: Cancer Research **2**:531, 1942.

3. Strong, L. C.: Am. J. Cancer **39**:347, 1940; Cancer Research **1**:572, 1941. Strong, L. C., and Williams, W. L.: ibid. **1**:886, 1941. Strong, L. C.; Collins, V. J., and Durand, E. A.: ibid. **3**:21, 1943. Strong, L. C.: Arch. Path. **36**:58, 1943. Williams, W. L., and Strong, L. C.: Cancer Research **4**:11, 1944. Strong, L. C.: Arch. Path. **37**:131, 1944.

4. Strong, L. C., and Smith, G. M.: Yale J. Biol. & Med. **13**:489, 1941.

5. Kirschbaum, A.: Proc. Soc. Exper. Biol. & Med. **55**:280, 1944.

nonbreeders on a diet of Nurishmix [5a] supplemented with a mixture of wheat and oats, enriched Bond bread [5b] soaked in milk and at intervals washed lettuce. Weekly observations were made on all animals, and as soon as it was apparent that the local tumor was definitely growing or as soon as any disorder was shown, such as emaciation, ruffled hair and wheezing, the involved mouse was killed. Within a few hours after death an autopsy was made on the mouse.

The present survey of tumors occurring in NH or NHO mice after the subcutaneous injection of methylcholanthrene is based on observations of 4,000 mice. The types of tumors observed included, among others, (1) fibrosarcoma, (2) rhabdomyosarcoma, (3) epidermoid carcinoma, (4) adenocarcinoma of the mammary gland, (5) adenocarcinoma of the pyloric end of the stomach, (6) adenocarcinoma of the lungs and (7) carcinoma of the liver. The study of the 4,000 mice (between the $F_4$ and the $F_{12}$ generation) was divided into two parts. The first part consisted of observations on 2,000 unselected mice (the NH) treated with methylcholanthrene; that is, every mouse of the early hybrid generations ($F_4$ to $F_8$) received an injection of the carcinogen. The second part consisted of observations on mice which were selected toward resistance to the induction of tumor at the site of the injection (the NHO mice, 2,000).

In testing for linkage (partially indicated by data obtained in the observations just mentioned) the hybrid mice of the NHO strain from the $F_{12}$ to $F_{16}$ generations have been divided into five sublines depending on the coat colors they carry. These are (*a*) brown nonagouti showing a high incidence of carcinoma of the stomach (both spontaneous and induced by methylcholanthrene), (*b*) brown nonagouti not showing carcinoma of the stomach, (*c*) pink eye brown nonagouti, (*d*) piebald brown nonagouti and (*e*) pink eye, piebald brown nonagouti.

## RESULTS

The gene on the "piebald"-tagged chromosome reduces susceptibility to the effects of methylcholanthrene by 10 to 15 per cent. (See chart 1. The rate of induction of tumors in self-colored mice [*a*] is represented by the short dash curve; the rate in piebald mice [*b*], by the solid line curve. The rate in $F_1$ [self-colored mice obtained by a cross between piebald and self-colored mice] [*d*] is shown in the open circle, long dash line. The rate in self-colored mice [*f*] obtained in the backcross to the recessive piebald stock is given in the solid dot, solid line curve and that in piebald mice [*e*], in the solid dot, long dash line.) In one descent of the brown nonagouti piebald subline this 10 to 15 per cent reduction of susceptibility no longer occurs. (In chart 1 the rate of induction of tumors in the "mutated" subline [*c*] is shown in the long dash line.) The stock is homozygous for "piebald." Consequently the phenomenon of "crossing over" cannot explain this loss of resistance to the local induction of tumors by methylcholanthrene. Further tests for linkage in the backcross to the recessive piebald subline have demonstrated that the new mutation has arisen, presumably at the site of the old gene (for local susceptibility to induction of tumors) on the "piebald"-tagged chromosome. That is, there is now no differential susceptibility between self-colored and piebald mice.

The second mutation has occurred on the X chromosome. The evidence is as follows: The analysis of the first 2,000 mice of the NHO descent shows that there is no sex difference in susceptibility to the local induction of tumors by methylcholanthrene. (See chart 2. The rate in the original females [*a*] is represented in the short dash line; that in males [*b*], by the solid line.) In one of the sublines there has appeared a sex difference. (In chart 2 the rate in the females of the "mutated" subline [*c*] is shown in a solid dot, solid line curve; that in the males of the "mutated" subline [*d*], in a solid dot, long dash curve.) In this subline the females are more susceptible to the local induction of tumors by methylcholanthrene than the males—a situa-

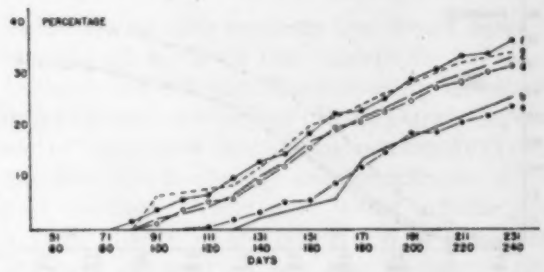


Chart 1.—Curves for the rates at which local tumors were induced when 1 mg. of methylcholanthrene was injected subcutaneously into (*a*) a homozygous Brown nonagouti self-colored subline of the NHO descent (short dash line), (*b*) a homozygous Brown nonagouti piebald subline (solid line), (*c*) the $F_1$ generation from a cross between "a" and "b" (long dash line), and (*d* and *e*) the two classes of self-colored and piebald mice obtained in the back cross to the recessive piebald subline (self-colored circle, long dash line, and piebald, solid dot, long dash line). Time in days is plotted along the base line; the percentage incidence of local tumors, on the vertical line (cumulative data). The curves indicate occurrence of a linkage between a gene of susceptibility to local induction of cancers and the piebald chromosome. Following this mutation (*f*) the derived piebald mice have the same susceptibility as the original self-colored mice (solid dot, solid line curve).

5a. Nurishmix, according to the manufacturer, contains: crude protein, not less than 20 per cent; crude fat, not less than 6.50 per cent; crude fiber, not more than 3.5 per cent; carbohydrates, 51.50 per cent; nitrogen-free extract, not less than 48 per cent. The ingredients are dried buttermilk, beef scrap, wheat germ meal, rolled oats, calcium pantothenate, molasses, iodized salt (1 per cent) and cod liver oil.

5b. Bond bread is stated by the manufacturer to contain: thiamine, 55 per cent of the minimum daily requirement of man; riboflavine, 17.5 per cent; nicotinic acid, 5 mg., and iron, 40 per cent of the daily human requirement.

tion that is also found in the CBAN stock. Tests for linkage in suitable genetic crosses have also disclosed the presence of this altered gene on the X chromosome.

That is, when mice of the original NHO strain (2,000 mice) were outcrossed to the $C_{57}$ black strain, there were obtained no sexual differences in susceptibility in the $F_1$ generation. This can be explained on the assumption that the genetic constitution of the old NHO strain as far as the X and Y chromosomes are concerned is the same as that of the $C_{57}$ strain—$X^{A\,1}$ $X^{A\,1}$ female NHO, $X^{A\,1}$ Y male NHO and $X^{A\,1}$ $X^{A\,1}$ female $C_{57}$, $X^{A\,1}$ Y male $C_{57}$—when the $A^1$ gene on the X chromosome has no effect on susceptibility. On the other hand, when mice of the altered or "mutated" NHO strain were outcrossed to mice of the $C_{57}$ strain there was a differential susceptibility between the sexes depending on the way in which the outcross had been made. When a female "mutated" NHO mouse was outcrossed to a $C_{57}$ male, the

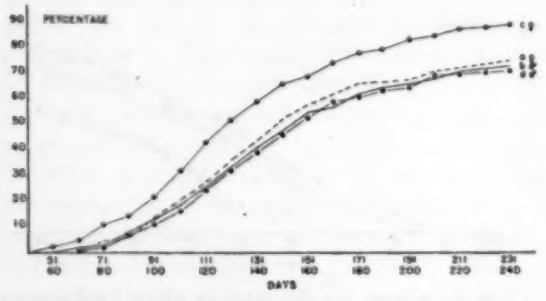


Chart 2.—Curves indicating a mutation occurring on the X chromosome, thus changing susceptibility to methylcholanthrene induction of tumors in the female but not in the male. Before the mutation arose there was no sex difference in susceptibility—(*a*) females, short dash line; (*b*) males, solid line—whereas after the mutation the females became more susceptible to local induction of tumors—(*c*) females, solid dot, solid line curve; (*d*) males, solid dot, long dash line curve.

$F_1$ males showed a higher susceptibility than did the females; whereas, when a male "mutated" NHO mouse was outcrossed to a $C_{57}$ female, the reverse was true. That is, the females now showed a higher susceptibility. These facts can be explained on the assumption that the genetic constitution of the "mutated" NHO strain is now $X^AX^A$ female, $X^AY$ male with A acting as a stimulator of local induction of tumors. The genetic constitutions of the $F_1$ mice would then be $X^AX^{A1}$ female, $X^AY$ male in the cross between a "mutated" female NHO ($X^AX^A$) and a $C_{57}$ male ($X^AY$) and $X^{A\,1}$ $X^A$ female, $X^{A\,1}$ Y male when the $C_{57}$ female $X^{A\,1}$ $X^{A\,1}$ was outcrossed to a "mutated" NHO male $X^AY$. The "A" gene acts as a stimulator of local induction of tumors whereas the "$A^1$" gene has no effect. In a third state the "a" gene acts as an inhibitor of susceptibility.[6]

*Classification of Data on Which the Curves in Charts 1 and 2 Were Computed*

| | Curve | Class | Mice |
|---|---|---|---|
| Chart 1 | - - - - | (*a*) Original self colored | 2,246 |
| | ——— | (*b*) Original piebald | 445 |
| | —— —— | (*c*) $F_1$ self colored × piebald | 158 |
| | o——o | (*d*) Self colored BC to b | 208 |
| | •——• | (*e*) Piebald BC to b | 219 |
| | •——• | (*f*) "Mutated" piebald | 351 |
| | | Total | 3,627 |
| Chart 2 | - - - - | (*a*) Original females | 485 |
| | ——— | (*b*) Original males | 507 |
| | •——• | (*c*) Females of "mutated" substrain | 194 |
| | •——• | (*d*) Males of "mutated" substrain | 179 |
| | | Total | 1,375 |
| | | Grand total | 5,012 |

## COMMENT

The sex-lined gene indicated by data from studies of the inheritance of susceptibility to methylcholanthrene induction of tumors is no doubt an "unstable" one, having apparently mutated at least twice before the establishment of the fifteen inbred strains developed by me and once since the introduction of methylcholanthrene. On the assumption that in one condition (A) the gene acts as a stimulator, in another ($A^1$) it has no effect on susceptibility, and in still another condition (a) it behaves as an inhibitor of the production of tumors, the genetic constitution of the mice of the various inbred strains are $X^AX^A$ female, $X^AY$ male in the CBAN stock, $X^aX^a$ female, $X^aY$ male in the CHI and C stocks, and $X^{A\,1}$ $X^{A\,1}$ female $X^{A\,1}Y$ male in the N, A, $C_{57}$, JKL, L, $C_{12}I$, FC, JK, CBA and $C_3H$ stocks. The present data indicate that the same gene has again mutated from $A^1$ to A following the injection of methylcholanthrene. The constitution of the new subline would then be $X^AX^A$ female and $X^AY$ male, the same as in the CBAN strain.

We are confronted with the problem of whether the two mutations were spontaneous or were induced by methylcholanthrene. The situation cannot be positively demonstrated. However, the impression is gained that the presence of methylcholanthrene within the body did have something

6. Strong, L. C.: Unpublished data.

to do with the appearance of two mutations within the relatively short period of six months. This impression is based on the following reasons: Germinal mutations in my laboratory have been extremely rare. In twenty-five years 210,000 mice have been raised and personally examined. During this period only eight germinal mutations have been detected. These were (*a*) albinism in the JK stock, (*b*) a recessive lethal "piebald" appearance in the CHI stock, (*c*) a "pink eye" in the C$_3$H stock, (*d*) absence of the xiphoid process of the sternum in the JK stock, (*e*) an extra claw on the front feet of JK mice, (*f*) polydactylism in 1 case in which inheritance was uncertain, (*g*) lop ear in the CHI—inheritance irregular—and (*h*) waltzing in the C$_{57}$ stock. So two mutations within six months seems to be more than should be expected from chance alone, especially when they were in the direction of increased susceptibility to tumor—a physiologic state already known to be influenced by methylcholanthrene somatically. The fact that no other germinal mutations have been reported by other investigators with thousands of animals receiving methylcholanthrene does not seem to invalidate the present interpretation when one takes into consideration that nearly all the work on the induction of tumors by methylcholanthrene in other laboratories has been done on animals and that pedigreed descendants have not been produced and kept (at least not reported). In fact most of the reported work was done specifically on mice not being used as breeders. Again the interest in using mice of the established inbred strains is rapidly gaining impetus. Now most germinal mutations are recessive, and one should not be able to detect them for several generations. My co-workers and I have deliberately used pedigreed hybrid mice and subjected their descendants for several generations to the same treatment of subcutaneous injection of methylcholanthrene. Under these conditions we have been able to detect two germinal mutations that influence susceptibility to the local induction of tumors. The fact that one of the genes which mutated, the one on the X chromosome, is apparently an "unstable" one should not weigh too much against the idea that the change (mutation) may have been enhanced by the presence of methylcholanthrene.

Mottram [7] briefly discussed the idea of carcinogens acting on genes (possibly during mitosis) rather than having a direct effect on living cells. He presented data on the appearance of chromosomal aberrations being produced in bean seedlings by carcinogenic tar. Unfortunately he did not distinguish between somatic and germinal mutations. I am not in agreement with Mottram's statement that "the mutation hypothesis for cancer postulates that the cancer cell differs from the normal cell in its content of genes."

In 1926 Strong [8] wrote:

> . . . changes within the genetic constitution of living forms, if they are germ cells, are called mutations; if they are somatic cells (occurring especially in plant tissues) they are called somatic mutations.
>
> The conclusion is therefore reached that somatic mutations may occur within the tumor cell, and that when these mutations do occur they change the reaction potential and other physiological activities of the tumor cell. The data at hand are not sufficient to determine more in detail the nature of this mutational process. It may be either a change or shifting of a complete chromosome or chromosomes, or a change or changes within a chromosome or chromosomes (genic or it may be even cytoplasmic in nature. By mutation I merely mean to use the term in its broadest sense, that is, a change or shift within the genetic or internal constitution that results in definitely clear cut or discernible differences in behavior or structure that is perpetuated by the process of heredity (in this case—cell division).

There are no data available that would necessitate the changing of this concept in any way. Certainly the new contributions on the mitochondria (Claude), the viruses (Rous, Duran-Reynals and others), extrachromosomal inheritance (Little and others) (Korteweg) and the influence of the milk (Bittner; Andervont) would indicate that a genic change in the conversion of a somatic cell into a cancerous one may be merely the final step and may not necessarily be the only one involved.

The cytologic or genetic changes involved in the origin of cancer under spontaneous as well as under induced conditions are shrouded in mystery. The present data on the appearance of two germinal mutations which underlie cancerous susceptibility are clearcut. The concept may be considered that the role of methylcholanthrene (and perhaps other carcinogens) in the induction of tumors has a twofold nature: (1) It has a direct effect on somatic cells and by some unknown process influences them to such an extent that they become cancerous (somatic mutations?), and (2) it also influences germinal mutations in a manner such that the constitutional states of susceptibility and resistance to cancer are altered and these changed states are transmitted to succeeding generations. The possible relationship of the somatic to the germinal changes in cancer is beginning to be indicated. In one type of neoplasm especially, adenocarci-

7. Mottram, J. C.: Brit. J. Exper. Path. **15**:71, 1934.

8. Strong, L. C.: Genetics **11**:294, 1926; J. Exper. Med. **43**:713, 1926.

noma of the stomach of the mouse,[9] this relationship seems to be a close one. That is, it has been demonstrated that the descendants of mice in which adenocarcinoma of the stomach has been induced by methylcholanthrene now continue to show development of the same condition spontaneously. Perhaps in other cancerous conditions, also, the role of the carcinogen may be of a dual nature—an effect on somatic cells and another somehow or other correlated change in the germ plasm. Either change may take place without the other one doing so.

SUMMARY

Two new germinal mutations which change susceptibility to methylcholanthrene induction of tumors have appeared in mice.

One of these mutations appeared on the "piebald"-tagged chromosome; the other, on the X chromosome.

The gene for susceptibility to induction of cancers on the X chromosome is apparently an "unstable" one, having mutated at least twice in the past and once following the subcutaneous injection of methylcholanthrene.

As to the role of methylcholanthrene in the induction of neoplasms, perhaps methylcholanthrene acts in a dual manner (1) directly on the somatic tissues, possibly bringing about somatic mutations, and (2) on the germinal mechanism which determines in part the intrinsic constitutional states of susceptibility and resistance to induction of cancers. The somatic and the germinal change may be, to some extent at least, correlated and influenced by the same extrinsic stimulus—in this case, methylcholanthrene.

9. Strong, L. C.: Rec. Genet. Soc. Am. **13**:39, 1944.

# CHANGES IN THE UTERUS AFTER ERADICATION OF ENDOMETRIAL ADENOCARCINOMA BY RADIOTHERAPY

## WITH PARTICULAR REFERENCE TO AN INFARCT-LIKE RADIONECROTIC PLAQUE IN THE LINING

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We [1] recently published a clinical report of the effects of combined roentgen and radium therapy on adenocarcinoma of the endometrium. In some of the cases hysterectomy was performed at varying intervals of time after the completion of the radiation treatments. Routine microscopic examination of grossly abnormal areas of the uterus in 5 cases revealed no evidence of carcinoma. In 4 of these cases (1 to 4) the uterus was later subjected to a much more thorough examination. There was still no evidence of carcinoma. However, various interesting changes induced by radiation were present, notably an infarct-like plaque of necrosis at the level of the internal os. It is the purpose of this paper to give a detailed description of the changes and to discuss the composition and the genesis of the plaque at the internal os. The observations in 2 other cases (5 and 6), which throw some light on these points, will be summarized also even though only routine sections were available for study.

### REPORT OF CASES

In the previous report [1] items of clinical importance were stressed. In the present paper only the most pertinent clinical data and the doses and the technics utilized in the radiation therapy will be furnished. Additional information can be obtained from the previous paper.[1] In tables 1 and 2 of that communication all 6 of the cases to be reported here are listed: The initials of the patients are used in the earlier report instead of the case numbers, as follows: R. B., case 1; P. G., case 2; D. C., case 3; M. S., case 4; A. D., case 5; A. M., case 6.

From the Department of Pathology and the Department of Obstetrics and Gynecology of Loyola University School of Medicine and the Mercy Hospital Institute of Radiation Therapy.

1. Schmitz, H. E.; Sheehan, J. F., and Towne, J.: Am. J. Obst. & Gynec. **45**:377, 1943.

CASE 1.—A woman aged 46 entered the hospital complaining that she had noted abnormal vaginal bleeding during the three years preceding entry. She had been pregnant five times and had borne 2 children. The fallopian tubes and ovaries had been removed six or seven years before. Microscopic examination of material removed from the uterus by curettage revealed adenocarcinoma of the endometrium (grade 3) (fig. 1). Combined roentgen and radium therapy was instituted. The total dose of radium was about 4,500 milligram hours; the total dose of roentgen radiation was about 4,000 roentgens (r) in the midpelvis. The uterus was removed three months after the completion of the treatment.

The uterus measured 10.4 cm. in length and 7 by 4.5 cm. at the fundus. The peritoneal covering was in part reddish tan and in part bright red and smooth except for several scattered torn red fibrous adhesions. The myometrium was about 2 cm. in thickness and contained one well demarcated mass of shiny white firm whorled tissue, 1.5 cm. in diameter, in the posterior wall near the left cornu. In the lower portion of the body immediately above the internal os and apparently extending down to involve the os there was a slightly raised mass with a flat, plateau-like top. This mass measured 2.5 cm. in length (in the long axis of the uterus) and completely encircled the lumen. It was composed of rather firm, yellowish white tissue replacing the endometrium and the adjacent myometrium to a depth of 3 mm. Its free aspect was covered by a thin layer of greenish yellow material. The endometrium elsewhere in the body was less than 1 mm. in thickness, smooth and tan or reddish tan.

The cervix was about 4 cm. in length and 3.5 cm. in diameter. The margins of the external os were slightly reddened and granular. Except for an occasional bright red patch the membrane covering the vaginal portion was normal. The cervical canal was lined by a smooth yellowish white membrane. The wall of the cervix was composed of pliable white tissue beneath a thin mucous membrane.

Routine sections were made and the absence of carcinoma established. Later three parallel transverse incisions passing completely through the uterus were made. These divided the uterus into four segments including the upper portion of the body, the lower portion of the body exclusive of the part adjacent to the internal os, the internal os and the portions of the body and of the cervix immediately adjacent, and the remainder of the cervix. Each of these four segments was further divided by longitudinal incisions (in the long axis of the uterus) so placed that in blocks about

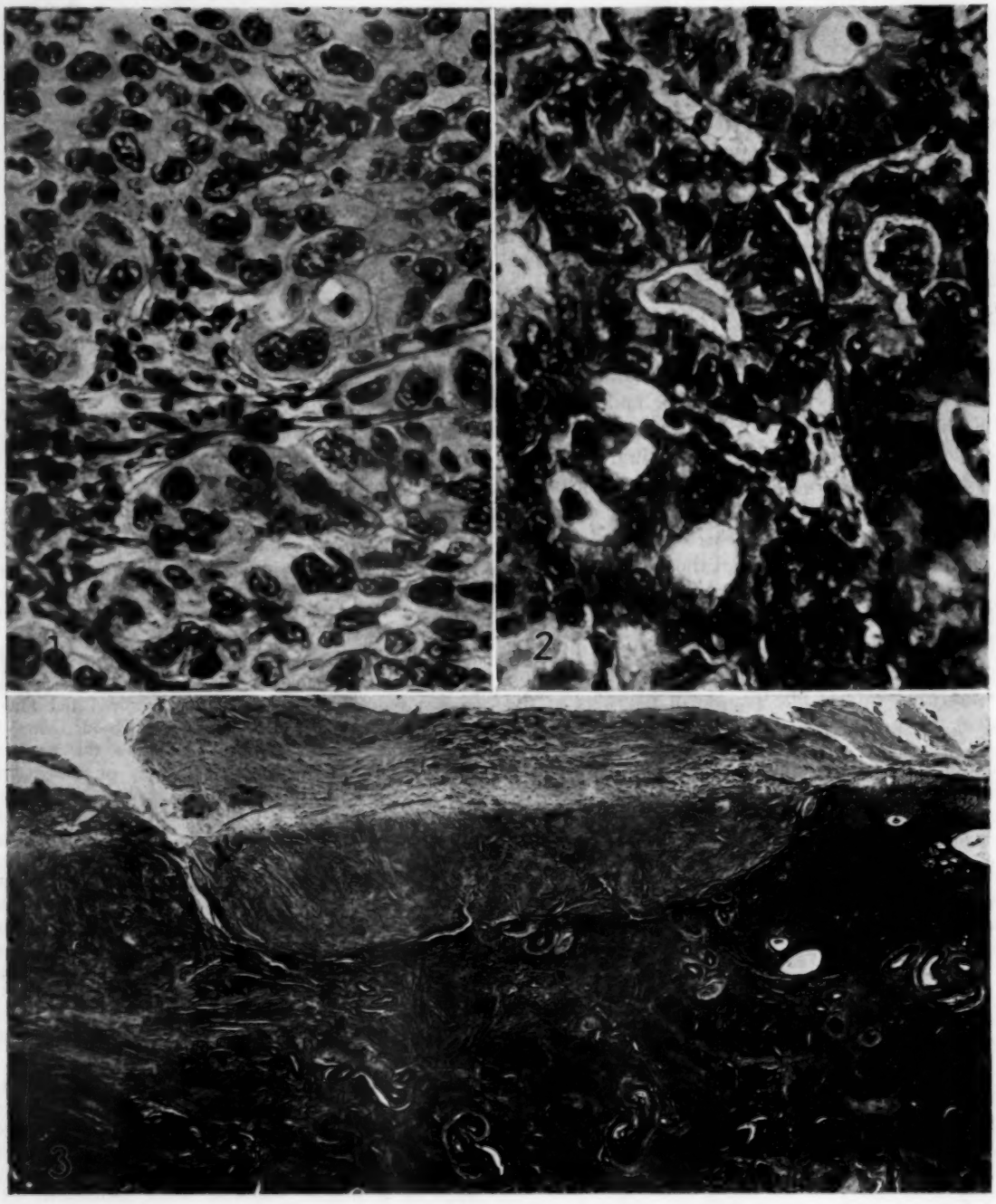


Fig. 1 (case 1).—Microscopic appearance of the adenocarcinoma of the endometrium eradicated by radiation therapy. All parts of the tumor included in the sections examined had a similar appearance. × 436.

Fig. 2 (case 3).—Microscopic appearance of the adenocarcinoma of the endometrium eradicated by radiation therapy. Most of the tumor was adenomatoid in type. In several areas, however, the pattern resembled that shown in figure 1. The structure of the tumor in cases 2 and 4 was varied. Patterns similar to those shown in figures 1 and 2 were revealed in each instance. In addition, in case 2 distinctly papillary areas were found. × 455.

Fig. 3 (case 2).—Typical radionecrotic plaque in the innermost part of the wall of the uterus. The plaque projects slightly into the uterine cavity above. It is well demarcated from the underlying myometrium. Except for changes in small arteries near the plaque, the myometrium is essentially the same adjacent to the plaque as at a distance from it. Note the absence of granulation tissue along the periphery of the plaque. × 16.

4 mm. in thickness all of the mucosa and as much as possible of the entire thickness of the wall were included. In the segments of the body of the uterus this was not feasible. Hence only the inner 1.8 cm. of the myometrium appeared in the blocks. Separate sections were made of the serosa and of the outer portion of the myometrium. The blocks were fixed in Bouin's fluid or in 4 per cent solution of formaldehyde. Paraffin sections from the surface of each block were prepared; then each block was cut approximately half way through, and additional sections were made. Thus the entire endometrium and endocervix with the tissues adjacent were sectioned at intervals no greater than 2 mm. The preparations were stained with hematoxylin and eosin. When indicated, Verhoeff's elastic tissue stain and Masson's trichrome stain for connective tissue were utilized.

*Microscopic Examination.*—(*a*) Upper Body: The endometrium was thin: the glands were few; some were cystically dilated. In some areas the epithelial cells on the surface were low or tall columnar, but for the most part they were cuboidal, with large round vesicular nuclei. Many of these cells had rounded free margins projecting somewhat into the uterine cavity, giving a scalloped appearance to portions of the surface. In some of these rounded cells vacuoles were found, often surrounding a pyknotic nucleus. Over large areas no epithelial covering cells could be seen. The epithelial cells lining the cystically dilated glands were flat or low cuboidal; those lining the more normal glands were for the most part markedly swollen and roughly cuboidal and contained large round or oval vesicular nuclei; the latter sometimes were arranged with long axes parallel to the lumens of the glands. Many of the nuclei were even more irregular. Many contained prominent nucleoli. In a few of the cystically dilated glands hemosiderin-laden macrophages or granular red or brownish red material was noted.

The endometrial stroma was fairly thin even where the endometrium passed outward for a short distance between muscle bundles. Between these prolongations it was particularly thin, often only five fibroblast-like cells thick. In areas in the most superficial portion of the endometrium there was definite edema; the connective tissue cells were flattened, elongated and separated by spaces. Their long axes were arranged parallel to the surface. Deeper the stroma cells were normal in appearance and were not abnormally separated. In a rare area a small irregular hyaline mass intermixed with red cells was noted in the endometrium. The overlying epithelial cells were in part swollen and basophilic; their long axes were parallel to the surface. Near the hyaline deposit the endometrium was edematous and infiltrated by numerous lymphocytes, monocytes and plasma cells, with fewer neutrophils and eosinophils intermixed. Elsewhere the endometrium either showed no cellular infiltration or was infiltrated by a few lymphocytes.

Near the small hyaline mass in the endometrium, some of the muscle bundles of the upper part of the myometrium were partially hyalinized. Among these, clumps of plasma cells and of red cells were found. Elsewhere the muscle bundles of the myometrium appeared normal or somewhat larger than normal. There was no increase in the connective tissue between them. A few small clumps of plasma cells were scattered throughout the myometrium in the section, even deep in it. A few small foci of leiomyoma were encountered; in the smaller ones the neoplasm was cellular, with nuclei closely set, owing to the small amount of cytoplasm in the individual cells, in the larger ones it was partially hyalinized.

Fairly numerous small arteries (137 microns or less in external diameter) with hyalinized walls were noted in the inner layer of the myometrium near the endometrium or in the lower layers of the endometrium itself. In a few areas in the vascular middle layer of the myometrium larger arteries (500 to 900 microns) showed marked swelling of the internal elastic membrane, the so-called "vitreous change," or an increase in elastic tissue in the adventitia. Sometimes in the relatively wide expanses of altered elastic tissue resulting from the degeneration of old larger arteries, new small arteries were found. These arterial changes have been considered indicative of subinvolution of the uterus (Schwarz [2]).

(*b*) Peritoneal Coat: Focal fibrous thickening of the connective tissues of the peritoneum covering the uterus was noted. Torn fibrous adhesions were attached in areas. Refractile material in contact with foreign body giant cells and a few lymphocytes were found in the fibrous tissues in the adhesions as well as in the foci of peritoneal thickening.

(*c*) Lower Body: In this segment there was noted a portion of the necrotic mass seen grossly at the internal os and in the adjacent portion of the body. The junction of this mass with the endometrium was also present in this segment. At the time of the gross examination this mass was interpreted as being the carcinoma revealed by curettage or the scar at the former site of a completely destroyed cancer. Microscopically it proved to be an area of coagulation necrosis simulating an infarct. In the descriptions which follow, this mass will be referred to as the plaque.

The central, thickest portion of the plaque consisted of rather finely granular, in areas faintly fibrillary, pale eosinophilic material abutting on the uterine cavity. In it were noted non-nucleated, ghostlike remnants of blood vessels and even more poorly defined outlines of muscle bundles. Some of the arteries were 300 microns or more in diameter. Some contained discrete red cells and were not thrombosed. In the deeper portion of this superficial zone a few neutrophils and many pyknotic muscle cell nuclei were present. This thick necrotic zone rested on altered myometrium. In the latter, two zones could be defined in areas, a deep zone of purely edematous myometrium with beginning atrophy of muscle fibers and a more superficial layer in which the muscle fibers were completely atrophic or necrotic and in which varying amounts of hyaline material were deposited. These two zones will be designated as the edematous myometrial zone and the hyaline-edematous myometrial zone respectively. Each was about 0.5 to 1 mm. in thickness. The two zones were not always sharply separated. They were best defined in the sections from material fixed in solution of formaldehyde in contradistinction to those preserved in Bouin's fixative.

The hyaline-edematous zone was usually, but not always, rather sharply marked off from the overlying zone of necrosis. In the hyaline-edematous zone only the nuclei of muscle cells presisted. These were often found in vacuole-like spaces. Around the blood vessels in this zone, and even around the vacuoles containing the nuclear remnants of muscle cells, irregularly distributed, faintly fibrillary sheets of hyaline material were deposited. In a few areas the deposits were dense enough to obliterate vacuoles and nuclei. Scattered giant fibroblasts and endothelial cells were present in

2. Schwarz, O. H.: Am. J. Obst. **79**:63, 1919.

the hyaline-edematous myometrial zone and less often in the edematous myometrial zone. Because of the disappearance of muscle bundles and the persistence of blood vessels, a false impression of a zone of granulation tissue in the more superficial portion of the hyaline-edematous myometrial zone was obtained. Close scrutiny indicated that there was no new formation of blood vessels. In fact, in many areas there was no suggestion of granulation tissue. There was a well defined transition from normal myometrium to edematous myometrium, from the latter to a partially hyalinized, edematous necrobiotic myometrium and finally from this hyaline-edematous portion to completely necrotic tissue. (Compare fig. 8 in a previous article.[3]) Over large areas there was no cellular infiltration in any of these four layers. Here and there in the hyaline-edematous myometrial zone (and even in the lower part of the completely necrotic layer) masses of red cells were seen. In the same areas there were deposits of hemosiderin or of hemosiderin-laden macrophages, sometimes accompanied by lymphocytes, monocytes and even eosinophils. Over the free surface of this portion of the plaque no epithelium was encountered, but there was a sharp line of demarcation from the lumen. At the junction of the plaque with the endometrium, however, the surface epithelium continued up over the plaque for a short distance. The epithelial cells here were swollen and basophilic; their nuclei were swollen and hyperchromatic, with the long axes parallel to the surface. A few neutrophils were noted in the plaque just beneath the desquamating epithelial cells. Near its junction with the endometrium the plaque was composed in areas of fused red cells, intermixed with neutrophils and fibrin, which penetrated beneath the surface epithelium of the adjacent endometrium and near the endometrium rested directly on the hyaline-edematous myometrial layer. Farther away from the junction of plaque and endometrium, as the thicker portion of the plaque was approached, the typical infarct-like necrotic surface layer replaced the fused red cell material. This, however, in places still remained superficial to the infarct-like zone of necrosis. Near the junction of the plaque with the endometrium in the hyaline-edematous myometrial zone, hyalinized necrotic muscle fibers, darker red than the other form of hyalin adjacent to them, were noted. Definitely identifiable hyalinized muscle fibers were noted only here in the margin of the plaque. Elsewhere the hyaline material could not be definitely traced to altered muscle cells but seemed rather to have been deposited there from the blood stream or to be altered blood clot.

Neither endometrium nor any identifiable portion of it appeared in the plaque except at the margin where the material of the plaque was continuous with similar material in the endometrium and where the surface epithelium continued from the endometrium up over part of the plaque. Elsewhere the endometrium was histologically similar to the endometrium in the upper portion of the body of the uterus.

The changes in the myometrium in contact with the plaque have already been described. Immediately beneath the intact endometrium there was mild edema of the myometrium. Occasionally a few monocytes were noted here. Deep in the myometrium beneath the plaque a few small clumps of lymphocytes and monocytes were situated near blood vessels or lymphatics. A few small foci of leiomyoma (0.1 to 1 cm. in diameter) were seen in this portion of the myometrium. In the larger ones, varying degrees of hyalinization and of early cystic change were observed. In the smaller ones, central hyalinization was not uncommon.

Marked changes were present in the arteries in the edematous and hyaline-edematous zones of the altered myometrium beneath the plaque. These changes consisted of swelling of endothelial cells, often with intimal edema and an infiltrate of lymphocytes, monocytes and even neutrophils and eosinophils; the presence in the intima of red cells, discrete or fused, of foam cell plaques[3] and of fibrin deposits; necrosis of portions of the vessel walls, particularly of the inner portions, and the presence of thrombi. The arteries involved measured 300 microns or more in diameter. The size of the involved arteries indicated that the plaque extended fairly deeply into the myometrium and possibly explained why hyaline intimal thickening and complete hyalinization of the arterial walls, well known effects of radiation treatment, were not common here. In fact, these hyaline changes were seen only in the arterioles in the innermost layer of the myometrium beneath the edge of the plaque.

Several myometrial arteries with long axes perpendicular to the surface were found running parallel to one another. As they passed through the edematous and hyaline-edematous myometrial zones into the zone of complete necrosis, the changes listed in the foregoing paragraph occurred and were largely limited to the portions traversing the altered myometrial zones or the tissues immediately adjacent. These changes were quite similar in the various arteries and were most pronounced near the zone of necrosis.

(*d*) Internal Os: The plaque was found throughout this segment, which included the portions of the body and of the cervix immediately adjacent to the internal os. An excellent opportunity was afforded to contrast the changes in the plaque and the adjacent tissues near the cervical termination of the plaque with those occurring in the uterine body near the upper termination of the plaque. Toward the uterine body the structure of the plaque and of the two subjacent altered myometrial zones and the type and the location of the arterial changes were the same as those reported in the description of the lower portion of the body of this uterus.

Toward the cervical side the hyaline-edematous zone was much more compact—in areas, in fact, not edematous at all. Superficial to it in areas was a granular necrotic zone, surmounted in turn by a layer of old blood clot. In the hyaline zone near the cervical termination of the plaque was a cervical gland with swollen, basophilic, poorly outlined, desquamating cells. Most of the nuclei in the gland were pyknotic.

Deep to this fairly dense hyaline zone, instead of a fairly wide edematous zone to correspond with the edematous myometrial zone there was a narrow zone of partially hyalinized fibromuscular tissue. In some of the slides this zone was continuous toward the cervical side with an area of loose structure filled with masses of red cells and hemosiderin, free or in macrophages. Plasma cells were abundant in the same area. Portions of dilated, partly necrotic arteries were seen in this same area. Some were filled with partially organized clot of an orange color; others contained discrete red cells. Hemorrhage undercut part of the endocervix at the lower margin of the plaque. There were masses of red cells and of plasma cells here. Elsewhere the endocervix was mildly edematous and infiltrated by a few lymphocytes. The glands in it

3. Sheehan, J. F.: Arch. Path. **37**:297, 1944.

near the plaque were composed of swollen, basophilic cuboidal cells with large round nuclei and did not secrete mucus. On the surface were a few cuboidal epithelial cells.

The edge of the plaque extended beneath the surface epithelium of the cervical canal in areas at least. The surface epithelium extending up over the edge of the plaque was composed of club-shaped cells, the free margins of which were ballooned out. Deep down in the wall of the cervix, extending from the area of recent and old hemorrhage, were clumps of plasma cells and of hemosiderin-laden macrophages, probably in or around lymphatics.

In contrast to the changes noted in arteries near the upper termination of the plaque in the body of the uterus, hyaline intimal thickening with or without occlusion and hyalinization of vessel walls were much more prominent in the cervical tissues immediately adjacent to the plaque and particularly near its lower edge. Here masses of hyalinized arteries were noted. Most of these were less than 300 or 350 microns in diameter. In addition, deeper in the wall of the cervix were notable vascular changes: dilated arteries with necrosis of parts of their walls; foam cell plaques; fibrin deposits in the intima; even hemosiderin and hyaline material in the intima. Occasionally fibroblastic intimal thickening was present. Some small arteries (about 100 microns) had completely hyalinized necrotic walls. The adventitia was edematous and infiltrated by lymphocytes, monocytes and occasionally by other cells as well.

(*e*) Cervix: The stratified squamous epithelium was absent in areas near the external os. Beneath one such denuded area there was hyalinization of a small portion of the fibromuscular coat. Masses of red cells, lymphocytes and monocytes infiltrated this area, and pronounced vascular changes, including necrosis of dilated arteries and hyaline necrosis of walls of other, undilated arteries, occurred.

Beneath the stratified squamous epithelium, as well as beneath the columnar epithelium, were well defined lymphoid follicles and, in areas, massive diffuse infiltration by lymphocytes and plasma cells. Fewer neutrophils and eosinophils were present. The epithelial cells of some of the cervical glands had swollen pale nuclei; others contained pyknotic nuclei. The fibromuscular coat was definitely not diffusely hyalinized.

*Summary.*—The observations included: small intramural foci of leiomyoma; atrophy of the endometrium; focal chronic endometritis; radionecrosis of part of the wall of the uterus at the level of the internal os; marked radiation changes in arteries near the area of necrosis; mild chronic metritis; marked chronic cervicitis; focal chronic perimetritis with foreign body giant cell reaction; no evidence of carcinoma.

Case 2.—The patient, aged 52, complained of vaginal bleeding during the six months preceding admission to the hospital. There had been three normal deliveries and no miscarriages. The menopause had occurred eight years before, and there had been no vaginal bleeding since until the onset of the present illness. Microscopic examination of uterine curettings revealed papillary adenocarcinoma of the endometrium (grade 2). Combined roentgen and radium therapy was instituted (6,000 milligram hours of radium and 4,000 r of roentgen radiation in the midpelvis). About five months after the completion of this treatment the uterus was removed.

The uterus measured 7.8 cm. in length and 4.8 by 3 cm. at the fundus. The serosa was smooth and glistening; its color was pinkish tan. The myometrium was about 1.4 cm. thick and composed of fibrillary, pale grayish tan tissue. At the level of the internal os there was an irregular mass on the mucosal aspect, 0.8 cm. in diameter, extending 0.5 cm. above the surrounding mucosa. This plaque was composed of soft grayish brown or pale greenish brown tissue. There was no gross extension of this tissue into the myometrium or the adjacent pale grayish white tissues of the cervical wall. Projecting into the uterine cavity from the endometrium of the fundal portion was a soft polypoid mass of pale grayish brown tissue, measuring 1.1 by 0.8 by 0.4 cm. Elsewhere the endometrium was pale pinkish tan and 1.6 mm. thick. The cervix measured 3.6 cm. in length and 3.4 cm. in diameter. The external os was mildly patulous; its margins, irregularly stellate. The mucous membrane of the vaginal aspect was smooth and pinkish gray except at the external os, where it was finely granular and pinkish tan.

The body of the uterus was divided longitudinally into two unequal halves. Microscopic examination of sections taken at that time revealed no carcinoma. Later the smaller unequal half was cut into numerous blocks by parallel incisions passing obliquely through the entire wall. The cuts were so spaced that all of the endometrium was contained on the 4 mm. thick edges of the blocks. Sections from this portion were labeled "½ Body." The rest of the uterus was divided into three transverse slabs labeled "Upper Body," "Lower Body" and "Cervix." These were later subdivided longitudinally and sections obtained in a manner similar to that in which sections were prepared in case 1.

*Microscopic Examination.*—The changes observed in the slides labeled "½ Body" and "Upper Body" were similar and will be described together.

(*a*) One-Half Body: Upper Body: The endometrium resembled that of the uterus in case 1. There was definite atrophy. The glands were reduced in number and widely separated. Some were cystically dilated. In the more superficial portion of the endometrium the well preserved fibroblast-like stromal cells were separated somewhat (mild edema). The epithelium in the glands and on the surface resembled that in case 1, but the constituent cells were not so swollen. Some of the cystically dilated glands contained red blood cells or hyaline globules. Some of the latter were large and resembled corpora amylacea. There were in areas a few lymphocytes, plasma cells, monocytes and red cells.

The inner half of the myometrium seemed much more compact than the outer half. In the inner half the muscle cell nuclei were much closer together than normally. This appearance was interpreted as indicating atrophy of these cells. At least the cytoplasm was reduced in volume. In the outer half there was considerable separation of the muscle and connective tissue elements, probably signifying edema. In the outer half, just beneath or extending into the serosa were a few calcospheroids and a few small glandlike spaces lined by mesothelium or epithelium. No endometrial stroma was noted around these glandlike structures. The serous coat was otherwise normal. A rare clump of lymphocytes was seen deep in the myometrium.

(*b*) Lower Body: In the middle coat of the myometrium, arterial changes characteristic of subinvolution (compare case 1) were observed. Near the endometrium there were only a few hyalinized, unoccluded arterioles.

In some sections a plaque similar to that in case 1 was in evidence (fig. 3). It was composed of a fairly wide zone of necrotic tissue in which the faint pattern of blood vessels, of muscle bundles and even of a few glands with swollen epithelium was still visible, particularly in the deeper portions. Masses of red cells were present in some parts. The necrotic zone was, however, much more compact, more hyaline-appearing than the corresponding zone in case 1. The necrotic layer was rather sharply demarcated from the underlying myometrium. No definite partially hyalinized, edematous myometrial zone was noted. In areas the necrotic material rested directly on apparently normal myometrium or myometrium in which only a few hyalinized muscle fibers could be seen. Elsewhere a narrow zone of edematous myometrium containing partially atrophied muscle fibers was present. Bizarre fibroblasts, few in number, were located in the lower part of the necrotic layer rather than in the edematous myometrial zone. The latter was infiltrated in a few areas by small numbers of lymphocytes, neutrophils, hemosiderin-laden macrophages, monocytes and eosinophils. A few small calcospheroids were also noted in the myometrium at the margin of the plaque. There were areas at the junction of the myometrium and the plaque where no cellular infiltration could be found.

Superficial to the infarct-like layer were masses of fused red cells (old hyalinized clot) with a few neutrophils and large calcified globules. The plaque extended into the adjacent endometrium at its margin. The surface epithelium of the latter extended up over the edge of the plaque. The epithelial cells in this extension were swollen and contained fairly large pyknotic or hyperchromatic elongated nuclei, the long axes of which were parallel to the surface.

Where the plaque met the endometrium a few lymphocytes and hemosiderin-laden macrophages were present in the endometrium.

In the myometrium adjacent to the plaque there were vascular changes. These consisted predominantly of hyalinization and thickening of the intima. In some arteries the media was swollen, hyalinized and even infiltrated by neutrophils. A few had swollen endothelial cells with or without lymphocytes and foam cells in the intima. Some arteries showed red cells in their walls. Rarely, mild fibroblastic intimal thickening was encountered. Deep in the myometrium a few small clumps of lymphocytes appeared in contact with the walls of veins or the lymphatics.

(*c*) Cervix: The plaque appeared in these sections also. Here, too, it was compact, rather densely hyaline but with a suggestion of whorled muscle bundles in its deeper part. A few necrotic blood vessels with discrete red cells in the lumens were found in it. The plaque was rather sharply demarcated from the adjacent viable fibromuscular tissue, which showed little if any hyalinization. In parts of the base of the plaque and in the adjacent fibromuscular tissues there were clumps of lymphocytes and hemosiderin. Elsewhere no cells were noted adjacent to the plaque.

Near the plaque the arteries showed mainly hyaline intimal thickening and varying degrees of hyalinization of the medial coat. Farther from the plaque, even deep down in the fibromuscular coat, arterial changes, much more varied, were seen, such as dilatation and necrosis, subendothelial presence of lymphocytes and fibrin, presence of foam cell plaques and intimal hyalinization. Clumps of lymphocytes, monocytes and eosinophils were found near blood vessels throughout the wall of the cervix.

Beneath the edge of the plaque cervical glands were identified. No cellular infiltration was seen in the poorly defined adjacent endocervical stroma. Some of the cervical glands at the edge of the plaque were dilated, contained thick mucus and were lined by flattened epithelium. Other dilated glands were lined by low columnar epithelium. Most of the surface columnar epithelium was replaced by a thin layer of fibrin and red cells. No other cells were found with the red cells.

*Summary.*—The observations included: an area of radionecrosis at the internal os with vascular changes in adjacent tissues; atrophy of the endometrium; partial atrophy of the inner half of the myometrium; mild chronic metritis; a completely necrotic polyp in the body of the uterus with a rare identifiable epithelial cell in it; chronic cervicitis; no evidence of carcinoma.

Case 3.—A woman aged 38 complained of vaginal bleeding, menorrhagia of one and a half years' duration, metrorrhagia of ten months' duration and severe hemorrhage occurring seventeen days before. She had been pregnant twice and had two children. By curettage a diagnosis of adenocarcinoma of the endometrium (grade 3) was established (fig. 2). A total dose of roentgen radiation of 4,000 r in the midpelvis was given in divided doses, along with a total dose of radium of 6,000 milligram hours. About eight months after completion of this therapy the uterus, tubes and ovaries were excised.

The uterus measured 10.7 cm. in length and 7.5 by 5.5 cm. at the fundus. The serosa was pinkish tan, smooth and glistening. The myometrium was 3.2 cm. in thickness, fibrillary and pale grayish tan. In the lower portion of the body was a plaque of soft, yellowish gray to yellowish tan tissue extending completely around the lumen. It rose about 0.4 cm. above the surrounding endometrium. The plaque was 3.3 cm. in length (in the long axis of the uterus). It extended downward into the upper part of the cervical canal. It extended outward into the wall of the uterus to a depth of no more than 0.5 cm. It was well demarcated from the myometrium.

The cervix measured 3.5 cm. in length and was 3 cm. in diameter, roughly globoid. The cervical os was patulous; its margins, stellate.

The uterus was cut into a multitude of blocks. The method of obtaining the blocks and the final individual slides has already been described in this paper (case 1). Just as in case 1, the entire thickness of the myometrium of the body could not be included in the same section with the endometrium; only the inner 1.5 cm. of the myometrium was retained with the endometrium. Separate sections of the serosa and the outermost portion of the myometrium were prepared.

*Microscopic Examination.*—(*a*) Upper Body: The endometrium was thin and contained few glands. The connective tissue cells of the lamina propria were thin and elongated, with long axes parallel to the surface. They were separated by spaces (edema), particularly in the more superficial portion of the mucosa. The endometrium was infiltrated by plasma cells with fewer monocytes, lymphocytes, eosinophils and hemosiderin-laden macrophages. In areas masses of plasma cells, often alongside masses of red cells, were seen. The few glands present were lined by flattened epithelium. Much of the surface epithelium was absent. Columnar epithelial cells and swollen cuboidal cells predominated in the areas still covered by epithelium. In the large cuboidal cells there were vacuoles, often con-

taining linearly compressed nuclei with long axes perpendicular to the surface. In areas some islands of stratified squamous epithelium with spines but without cornification were identified. In other areas low cuboidal cells with deeply basophilic elongated nuclei with long axes parallel to the surface were present. In a few areas the free borders of the surface epithelial cells were convex toward the lumen, giving a scalloped appearance to the free surface of the epithelium.

Except near the endometrium, the myometrium was composed of normal or possibly of somewhat enlarged muscle fibers, arranged in bundles. Between these there was a moderate increase in the amount of connective tissue. Some of the muscle fibers near the endometrium were mildly hyalinized and separated a little from one another by small spaces (edema?). In a rare area there was marked hyalinization of some of the muscle bundles near the endometrium. Here considerable plasma cell infiltration was noted. Clumps of plasma cells were also seen somewhat deeper in the myometrium.

There was complete hyalinization of many of the small arteries (100 microns or less in diameter) in the myometrium near the endometrium. Some of these were occluded. In larger arteries, only the internal layers of the wall were hyalinized.

The serosa was essentially normal.

(*b*) Lower Body: The plaque noted during the gross examination was found in all of the section of this segment of the uterus. No endometrium was present.

The plaque was composed over large areas of a fairly thin surface layer of fibrin and neutrophils and immediately subjacent, a layer of old hyalinized blood clot (or fused red cells). Deep to this lay a zone of partially hyalinized, edematous, necrobiotic myometrium (fig. 4) and finally, still deeper, a zone of edematous myometrium with some degree of atrophy of the individual muscle cells. The last two zones were 0.5 to 1 mm. thick respectively. In the hyaline-edematous zone near the old clot giant bizarre fibroblasts and endothelial cells were so prominent as to give the impression of definite granulation tissue extending into the clot (fig. 4). There did seem to be some increase in the number of capillaries here, and so it is probable that true granulation tissue was present. The hyaline material in this zone was paler than that in the old clot adjacent and seemed to be deposited along capillaries in particular, as well as along individual necrotic muscle fibers. It was difficult to decide whether or not this hyalin was not part of the denser old hyaline clot adjacent to it. Sometimes in this zone definitely hyalinized, not swollen, muscle cells with fading nuclei were seen but they were not numerous.

The surface clot was present throughout all of the sections. There were no ghosts of former endometrial or myometrial structures in the surface necrotic layers.

For the most part there were no inflammatory cells in the edematous myometrial zone or in the lower part of the hyaline-edematous zone. In the upper part of the latter zone, however, there was usually mild to moderate infiltration by lymphocytes and monocytes, with fewer neutrophils, eosinophils and hemosiderin-laden macrophages (fig. 4). Red cells in masses were not uncommon in this zone. In a few parts of this zone masses of plasma cells and of hemosiderin occurred and seemed to overflow into the adjacent edematous myometrial zone or even into the neighboring more normal myometrium.

The fibroblasts and even the endothelial cells of the capillaries in the upper part of the hyaline-edematous zone were most bizarre; they were spindle shaped, triangular, stellate or even more irregular; they had dark cytoplasm and giant nuclei, single or multiple. The giant nuclei were often hyperchromatic, sometimes pyknotic, appearing as dark basophilic smudges, at times even coarsely vacuolated. The cytoplasm of the same cells in some instances contained vacuoles or even neutrophils.

Down through the myometrium beneath the plaque, clumps of lymphocytes and monocytes were observed near vascular channels.

Vascular changes were most prominent in the edematous myometrial zone and consisted mainly of complete or partial hyalinization of the walls of very small arteries (100 microns or less), sometimes with occlusion, and intimal hyalinization in larger arteries. In the hyaline material in some of the hyalinized vessels pale areas or vacuoles could be seen.

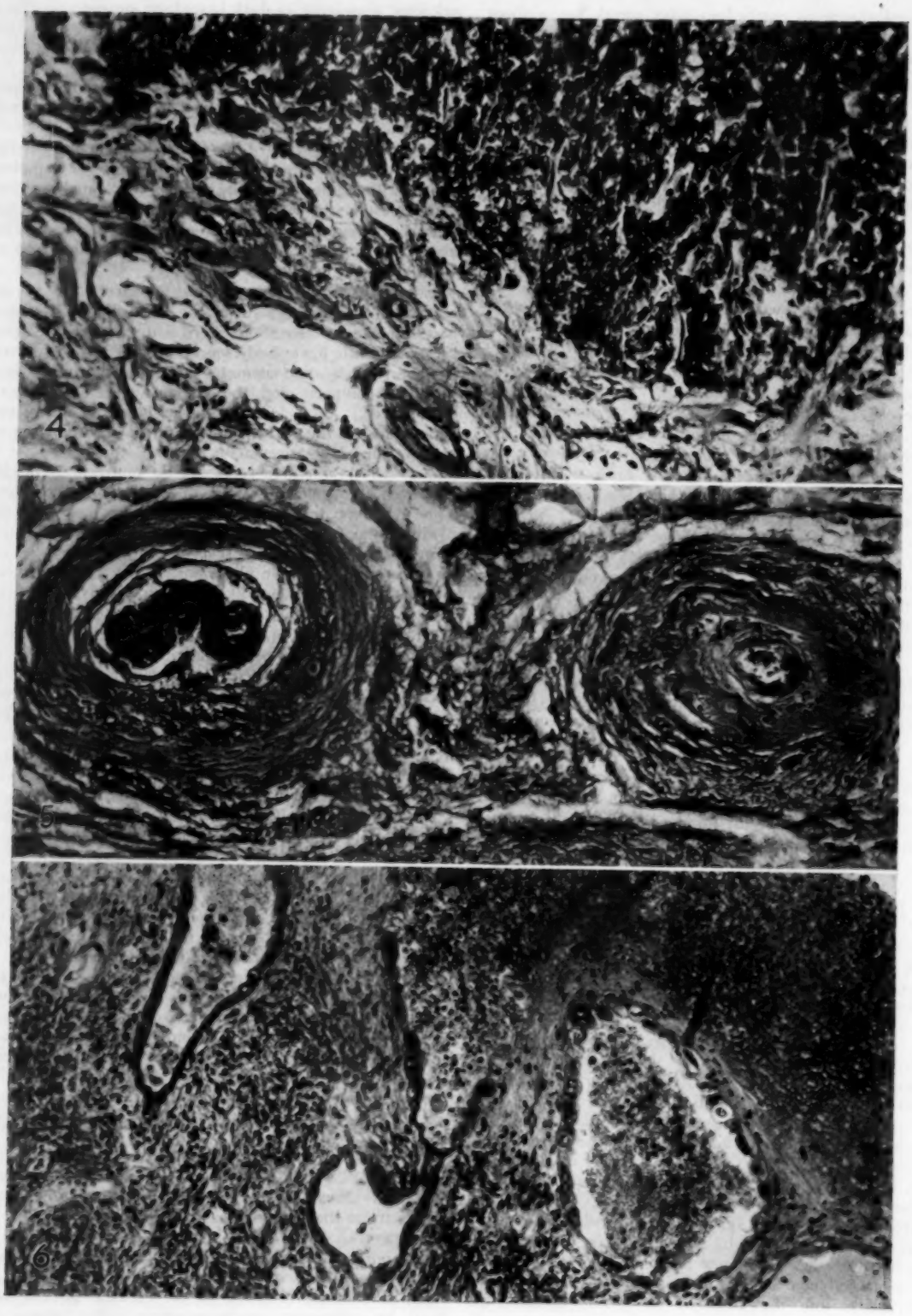
(*c*) Internal Os: The plaque appeared also in the sections from this segment of the uterus. For the most part, the tissues beneath it were composed of the same layers as in the lower body segment. Toward the cervix, however, the hyaline-edematous zone became more hyaline and compact, and the subjacent edematous zone disappeared. In a few areas the layer of fibrin and neutrophils on the surface dipped down into the layer of old hyalinized clot, replacing varying thicknesses of it and in at least one area replacing the entire layer of clot and even the uppermost part of the hyaline-edematous zone. Where this event occurred there were in the hyaline zone definitely hyalinized muscle fibers, much more numerous than were seen in any other area. Some of these hyaline muscle fibers had faintly staining nuclei and appeared somewhat like partially autolyzed fibers.

A second change was noted in this same area: fibrinoid necrosis of walls of small blood vessels. In other words, the walls seemed to be composed entirely of fibrin or fibrin-like material, internal to which in areas swollen endothelial cells could be seen. Discrete red cells were present in some of these vessels; clots, in others. Toward the body of the uterus the arterial changes were most pronounced in the edematous myometrial zone and consisted mainly of hyalinization. Toward the cervix, even though still in the myometrium, the arterial changes were more diversified. Dilated, partially necrotic arteries with hemosiderin around them were noted in the edematous myometrial zone or on either side of it. Massive plasma cell infiltration occurred here, along with the hemosiderin. Other changes included: hyaline deposits in the intima, fibrin, or lymphocytes, monocytes and other cells in the intima, foam cell intimal plaques and even hyaline deposits mixed with hemosiderin in the intima. Where fibrin and neutrophils replaced the layer of old clot and the upper part of the hyaline-edematous zone, fibrinoid necrosis of blood vessels ensued in the hyaline zone, and the changes already described in the larger arteries occurred in the subjacent tissues. In general, toward the cervical side arterial changes were located at greater distances from the necrotic plaque than they were on the uterine body side.

Throughout the wall of the cervix, adjacent to the plaque, small clumps of cells, mainly plasma cells, could be found along the course of blood vessels. In the myometrium the clumps were composed predominantly of lymphocytes.

The portions of endocervix seen in these sections were covered by a layer of stratified squamous epithelium instead of columnar. Some of the cervical glands were lined by columnar, mucin-secreting cells; others, by swollen columnar cells which did not secrete mucus.

(*d*) Cervix: There was no evidence of a plaque in any of the sections labeled "Cervix." However, the canal was lined by stratified squamous epithelium, beneath which accumulations of plasma cells and lymphocytes



FIGURES 4, 5 AND 6

*(See legends on opposite page)*

with a few neutrophils and eosinophils could be found. Where the stratified squamous epithelium was particularly thin, well defined lymphoid follicles had formed. In areas beneath the stratified squamous epithelium covering the vaginal aspect, some focal hyalinization of the fibromuscular coat and accumulations of red cells were found.

There was no hyalinization of the connective tissues around the cervical glands. Some of the latter were lined by columnar, mucus-secreting cells; others, by swollen cuboidal epithelial cells with large nuclei. A few glands were cystically dilated.

Toward the upper end of the portions of the cervical canal which appeared in these sections numerous arteries showed the effect of radiation and were similar to those described under the heading "Internal Os". Even thrombosed arteries were encountered. Along the blood vessels or lymphatics in the wall of the cervix were clumps of plasma cells with lymphocytes or monocytes or both intermixed.

*Summary.*—The observations included: an area of radionecrosis at the level of the internal os with marked vascular changes in the adjacent tissues; atrophy of the endometrium; focal squamous metaplasia of the columnar epithelium of the endometrium; chronic endometritis, chronic metritis; diffuse fibrosis of the myometrium; possibly mild muscular hypertrophy of the body of the uterus; marked chronic cervicitis; bilateral chronic salpingo-oophoritis and perisalpingo-oophoritis; small left hydrosalpinx; atrophy of the ovaries; no evidence of carcinoma.

CASE 4.—An unmarried woman, aged 65, consulted her physician because of an odorless, watery, at times blood-tinged vaginal discharge which had been present for three months. The menopause had occurred twelve years before. The curettement revealed adenocarcinoma of the endometrium (grade 2). The standard radiation therapy was given—radium, 6,000 milligram hours; roentgen radiation, 4,000 r, in the midpelvis. A hysterectomy was made about four months after the therapy.

The uterus was received in five pieces. The largest of these measured 5.3 by 3.6 by 2.5 cm. and apparently comprised the greater portion of the body. Another piece, measuring 4.5 by 3 by 1.6 cm., included all of the cervix. The external os was widely dilated; the margins were thin, dark grayish red or reddish tan. The cervical canal was lined by friable, dark greenish gray or reddish gray material, 1 mm. thick. Where identified, the serosa over the body was pinkish tan and smooth. Over large areas it was absent, and shaggy torn myometrium was exposed. The endometrium in the body was rough, somewhat shaggy, greenish gray to reddish gray and thickened in one area, where it measured 0.6 cm. in diameter. The myometrium was pale grayish tan, fibrillary and 1.4 cm. in thickness. In it several firm, well demarcated masses of glistening, grayish white whorled tissue were found. A mass of similar tissue, 2 by 1.5 by 1 cm., was received separately. The central portion of this mass was calcified. Finally, a separate strip of peritoneum and smooth muscle, apparently from a cornu of the uterus, was also received.

The largest portion of the uterus received, apparently the major portion of the body, was cut into two transverse slabs, each of which was divided by numerous longitudinal incisions into blocks about 4 mm. thick, which included the entire wall. Since it was impossible to be certain which portion of the mass was the fundus and thus to orient the slabs, the sections from them were labeled "Body 1" and "Body 2" respectively. The cervix was divided by longitudinal incisions into blocks which included the entire wall. Slides were made from these blocks in the same manner as in the preceding cases.

*Microscopic Examination.*—(*a*) Body 2: A plaque, somewhat similar to those described in the previous cases, was found in the sections from this segment. The most superficial layer was made up of old hyalinized clot with many neutrophils. In areas much fibrin was intermixed. In the myometrium beneath this layer a fairly wide hyaline-edematous zone, not sharply demarcated from an underlying edematous zone, was seen. In the hyaline-edematous zone and lower portion of the adjacent clot, fibrinoid necrosis of small vessels was apparent. In this and the adjacent edematous myometrial zone there were areas of only mild infiltration by lymphocytes, monocytes, neutrophils, plasma cells and eosinophils. Elsewhere the infiltration by these cells was much more pronounced; and considerable hemosiderin, free or in macrophages, and numerous red cells were often intermixed. In some areas beneath the surface clot a thin zone of edematous myometrium and atrophic muscle fibers were particularly heavily infiltrated by these cells. Here also several bizarre giant fibroblasts were seen. In the areas where a definite hyaline-edematous zone was in evidence, definite transitions from normal muscle fibers to thin atrophic muscle fibers to naked muscle cell nuclei could be traced. As the stage of naked nuclei was approached, there was increasingly larger deposits of hyalin around them. This hyalin was pale in contrast to the darker red hyaline material in the adjacent clot.

EXPLANATION OF FIGURES 4, 5 AND 6

Fig. 4 (case 3).—Hyaline-edematous myometrial zone (pale lower part of field) at the junction of the myometrium with the plaque. The infarct-like area of necrosis of the typical plaque (fig. 3) is replaced by masses of fused red cells or old clot (the dark material in the upper part of the field, particularly the right upper corner). Note the pale hyaline material around blood vessels and along necrobiotic muscle fibers in the hyaline-edematous zone. There is a definite suggestion of granulation tissue here and mild infiltration, the infiltrating cells being predominantly lymphocytes and monocytes. × 205.5.

Fig. 5 (case 4).—Small arteries deep in the wall of the cervix. In the artery to the left a somewhat folded loop of fibrin (dark band) surrounds the narrowed lumen. There are edema and deposition of hyalin in the intima and the inner part of the media with a few neutrophils in the hyaline material. There are large deposits of hyalin in the intima and the media of the artery in the right half of the field. There are no identifiable endothelial cells around the narrow lumen.

Fig. 6 (case 6).—Necrosis of the superficial portion of the endometrium with deposition of fibrin and massive neutrophilic infiltration. The space in the right upper corner represents the uterine cavity. The stroma of the deeper portion of the endometrium, around the bases of the glands, is edematous and infiltrated by lymphocytes, plasma cells and monocytes as well as by neutrophils. Note the radiation changes in the epithelial cells of the endometrial glands (nontumorous). A small portion of the myometrium appears in the lower left corner of the field. The muscle fibers show little deviation from the normal. × 156.5.

The endometrium throughout the body, even at a distance from the plaque, was completely replaced by a layer of neutrophils and old hyalinized blood clot except in a rare small area. Sometimes the innermost portion of the myometrium was likewise replaced by this material. In the tissues adjacent to this necrotic surface layer were numerous plasma cells, monocytes and hemosiderin-laden macrophages, as well as hemosiderin deposits. In some spots the cellular infiltration and the deposits were massive. The rare island of surface endometrium identified was thin and showed loss of much of the surface epithelium. A few groups of mildly swollen, pseudostratified columnar cells were still attached. Only a rare gland was seen. These were cystically dilated and lined by flattened epithelium. The stroma was composed of attenuated, elongated connective tissue cells, rather widely separated (edema). The stroma was diffusely infiltrated by lymphocytes and plasma cells. Numerous red cells were present.

nuclei. Other glands were cystically dilated and lined by flattened epithelium. Some of the glands contained monocytes and lymphocytes or monocytes and red cells in their lumens. The stroma of the ectopic endometrial tissue was in some areas compact, in others edematous or somewhat atrophic but not fibrotic. In the stroma in some of the islands of ectopic tissue plasma cells and hemosiderin were encountered. In the myometrium beneath the plaque small clumps of lymphocytes were located along lymphatics or blood vessels. In the upper edematous, partially hyalinized myometrial zone and in the surface zone of complete necrosis with old hemorrhage, fibrinoid necrosis of small blood vessels occurred. Deeper, even in the more normal myometrium, but more often in or near the edematous myometrial zone were a few hyalinized small arteries and also arteries with cells and fibrin or foam cell plaques in the intima. There was only a rare hyalinized arteriole near the endometrium.

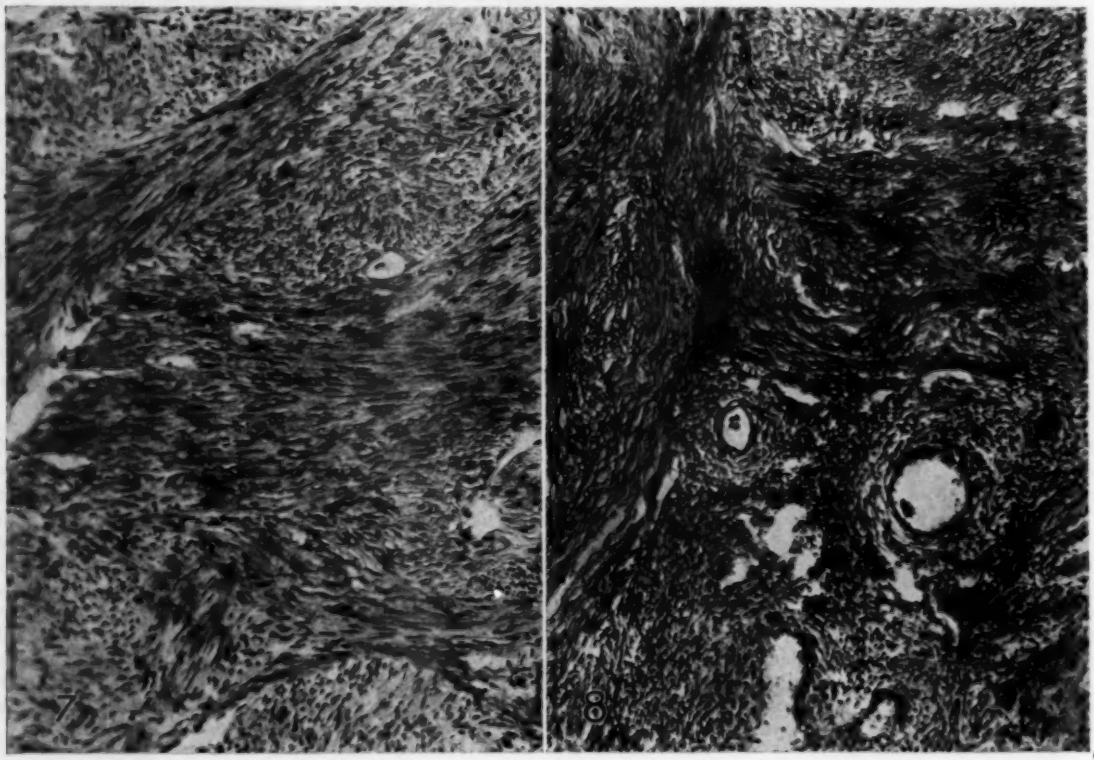



Fig. 7 (case 1).—Representative section of the myometrium. There is no muscular atrophy. In fact, the muscle fibers in the section from which this photomicrograph was made seemed somewhat larger than normal. × 125.

Fig. 8 (case 4).—Atrophic ectopic endometrium in the myometrium (in the lower half of the field) twelve years after the menopause and four months after the completion of radiation therapy. × 125.

The muscle bundles of the myometrium were of normal size. The individual muscle cells showed little deviation from the normal except near the endometrium or near the surface necrotic layer, where hyalinization and even some gradual necrosis occurred. Here there was infiltration by the cells already previously listed, particularly by plasma cells. In the myometrium small foci of leiomyoma were seen, some partially hyalinized. In addition, numerous islands of ectopic endometrial tissue were identified beneath the plaque and elsewhere as well (fig. 8). Those beneath the plaque were composed predominantly of endometrial stroma. However, a few glands were seen in this location. These contained swollen cuboidal epithelial cells with large vesicular

(*b*) Body 1: Another portion of the plaque just described appeared in the sections from this segment of the uterus. Much of the plaque had the same composition as the portion described already. However, in one area the whole top of the plaque appeared autolyzed and was teeming with bacteria. Beneath this bacteria-laden layer, a mass of fibrin and neutrophils rested directly on a wide zone of markedly edematous myometrium, which extended almost to the serosa. There was massive diffuse neutrophilic infiltration of almost the entire uterine wall here. In that part of this edematous myometrium near the layer of neutrophils and fibrin, definite groups of hyaline muscle fibers or muscle fibers showing gradual attenuation could be

observed. Vascular changes in this region were not notable. Those which occurred consisted mainly of edema of the entire arterial wall with mild infiltration by neutrophils, particularly in the intima. A few arteries at the edge of the plaque were thrombosed. Toward the cervix, arteries with hyaline intimal thickening could be found but even here there were a few neutrophils in the walls. In general, greater neutrophilic infiltration was found in arteries here. Fibrin and red cells were noted in the outer myometrium near the serosa in one region and clumps of lymphocytes and plasma cells near it in other areas. No fibrin was seen on the serosa.

(*c*) Cervix: No mucous membrane was identified anywhere; it was replaced by a layer of necrotic, amorphous debris and fused red cells or old hyalinized clot. This passed directly over into the fibromuscular layer. The latter near the surface was hyalinized in spots and rather massively infiltrated by monocytes, lymphocytes and plasma cells with fewer neutrophils and eosinophils. Small clumps of plasma cells were found deep in the cervix.

Vascular changes were marked, particularly near the upper end of the canal. Fibrin and cells appeared in the thickened intima of some arteries; hyaline necrosis and neutrophilic infiltration of the walls took place in others. Thrombosis was not uncommon. Acute diffuse inflammation of the walls of small veins was also seen.

*Summary.*—The observations included: Acute gangrenous endometritis and endocervicitis with extension of the inflammatory process into the adjacent myometrium and the fibromuscular coat of the cervix in areas; a plaque of radionecrosis at the level of the internal os with secondary bacterial invasion; chronic metritis and cervicitis; acute diffuse metritis near the internal os; radiation changes in blood vessels near the plaque; multiple small intramural foci of leiomyoma, ectopic endometrium in the myometrium; no evidence of carcinoma.

Case 5.—A patient aged 50 entered the hospital complaining of a thick white vaginal discharge and of vaginal bleeding. The white discharge had been present for one month; the bleeding, for ten days. Menstruation had ceased five or six years previously, and since that time there had been no vaginal bleeding until ten days before entry. The patient had five children. There had been no miscarriages. The uterus was curetted and a diagnosis of adenocarcinoma of the endometrium (grade 2) established. Radiation therapy included both radium and roentgen radiation (radium, total dose, 6,000 milligram hours; roentgen radiation, 4,000 r in the midpelvis). The uterus, tubes and ovaries were removed about four months after the radiation treatments were completed.

The uterus was 9 cm. in length and 6 by 4.5 cm. at the fundus. The peritoneal covering was smooth and reddish tan. The entire endometrium and that portion of the myometrium immediately adjacent were replaced by a foul-smelling, shaggy, green membrane to which, in the left cornu, a putty-like mass of necrotic, darker green tissue was attached. This mass measured 2 by 1 by 1 cm. The myometrium varied in thickness from 1 to 1.5 cm. It was white and glistening in its outer half. Nearer the necrotic lining the myometrium was pale green. A darker green layer of myometrium, 3 mm. wide, adjoined the dark green necrotic lining. The cervical canal was lined by a red, soft membrane, beneath which the pliable white tissues of the fibromuscular coat could be seen.

Several blocks were taken from various parts of the uterus and fixed in 4 per cent solution of formaldehyde or Bouin's fluid. Paraffin sections were stained with hematoxylin and eosin.

*Microscopic Examination.*—The entire endometrium and endocervix were replaced by a layer of necrotic debris. In the body this was rather wide. It was composed of amorphous, poorly staining material in which masses of bacteria could be seen. The lower part of this layer was densely infiltrated by neutrophils, deep to which was a partially hyalinized zone of edematous myometrium, rather heavily infiltrated by neutrophils. This was not sharply demarcated from a wide zone of underlying edematous myometrium. The edema in areas extended almost to the serosa. It was accompanied by some neutrophilic infiltration. Near the serosa small clumps of lymphocytes, plasma cells and monocytes were seen.

In the hyaline myometrial zone there were definitely hyalinized, palely staining muscle fibers. The hyaline material in this zone was very pale. In this same zone, as well as in the necrotic zone above it, fibrinoid necrosis of small blood vessels had occurred. Infiltration of the swollen intima of some arteries by neutrophils and even complete necrosis of parts of the walls of arteries with thrombosis were seen, particularly where the necrotic surface layer extended deep enough to involve parts of the walls of larger arteries.

In the edematous myometrial zone there were a few giant endothelial cells and fibroblasts. Swollen endothelial cells appeared in small arteries and were often associated with intimal edema and deposits of fibrin or even neutrophilic infiltration.

On portions of the serosa a thin layer of fibrin and a few neutrophils could be seen.

Deep to the necrotic material and neutrophils lining the cervical canal lay a thin layer of hyalinized fibromuscular tissue heavily infiltrated by plasma cells and lymphocytes. Near the upper end of the cervical canal, the hyalinization of the fibromuscular coat extended deeper, and a hyaline clot appeared on the surface. Arterial changes were marked here, with hyaline intimal thickening, often massive, and thrombosis in evidence. Deep in the cervical wall here similar arterial changes could be seen.

*Summary.*—The observations included: acute gangrenous endometritis and endocervicitis with extension of the inflammatory process into the adjacent myometrium and cervical wall; acute diffuse metritis, marked near the necrotic lining; a completely necrotic polypoid mass in the left cornu; chronic cervicitis; mild focal acute perimetritis; mild acute perisalpingitis; atrophy of the ovaries; no evidence of carcinoma.

Case 6.—A patient aged 42 complained of pain in the back and of a greenish vaginal discharge which had been present for six months. The menstrual periods had always been irregular, but they had been particularly so during the six months preceding entry into the hospital. A diagnosis of adenocarcinoma of the endometrium (grade 2) was made on examination of material obtained by curettement. Radium therapy was begun. The patient had received a dose of only 1,800 milligram hours when her condition indicated that the treatment should be stopped. No roentgen radiation was used. About one month later the uterus and adnexa were removed.

The uterus measured 9 cm. in length and 7 by 4.5 cm. at the fundus. The peritoneal surface was smooth, glistening and pinkish yellow with dark red patches. The myometrium was 2 cm. thick, yellowish white and fibrillary. The enodmetrium was thin (about 1 mm.). At the level of the internal os a dark red, slightly elevated portion of the lining, 0.4 cm. in diameter, was seen. The cervix was 3 cm. in length and somewhat enlarged

and boggy. Beneath the membrane of the cervical canal there was one well demarcated nodule of shiny white whorled tissue, 0.5 cm. in diameter.

Several blocks were removed from the uterus and fixed in Bouin's fluid. Sections were prepared as in the preceding case.

*Summary of the Microscopic Examination.*—Over large areas the endometrium and the innermost part of the myometrium were replaced by a layer of neutrophils, blood and debris. The superficial portion of the adjacent myometrium was hyalinized. No definite zone of edematous myometrium was seen. The hyalinized zone was widest and the clot on the surface thickest at the internal os. In the hyalinized zone, muscle fibers apparently undergoing gradual hyalinization were seen. It was difficult, however, to decide whether they were being hyalinized or whether hyaline material was being deposited around the nuclear remnants of atrophic or necrotic muscle cells. Where the surface layer of necrotic material and blood was thinnest there occurred considerable plasma cell infiltration with milder neutrophilic infiltration. Hemosiderin was present too. Where the hyalinized myometrial layer was very thin, no definite changes in the small arteries except fibrinoid necrosis could be seen. Where this layer was thick, various other arterial changes were noted: swelling of the endothelial cells, with fibrin or neutrophils or foam cell plaques in the intima. The rest of the myometrium was normal. No tumor was found in it.

In areas the endometrium was still present. A layer of fibrin or of fibrin and red cells with a few neutrophils overlay some parts of the surface epithelium and extended from the mouth of one gland to that of the one adjacent. Similar material was found in the lumens of the glands. The surface epithelial cells beneath the fibrin were swollen, glassy, or finely granular and deeply eosinophilic. Some of the nuclei were large and vesicular or hyperchromatic; some were small and deeply basophilic (pyknotic). Beneath this altered epithelium in areas plasma cells and hemosiderin were seen; in other areas red cells, fibrin and dilated capillaries were present. Edema was in evidence, but the connective tissue cells of the stroma appeared normal. Here and there the surface epithelium was absent. In such areas the fibrinous pseudomembrane on the surface was attached. At such points blood clot, partially hyalinized, was often seen. Similar small clots, recent or old, were located beneath other parts of the surface epithelium and caused some protrusion of it into the lumen. The epithelial cells overlying the clots were elongated and flattened and had elongated dark nuclei parallel to the surface.

The epithelial cells in the glands were quite varied in appearance. Many were large with large vesicular nuclei, round, oval, indented and lying parallel or at right angles to the lumen. Some were so swollen that their free borders projected into the lumen. Where the most superficial part of the endometrium, including parts of the glands, was replaced by fibrin or fibrin, red cells and neutrophils, the remnants of the glands were often lined by epithelial cells which differed greatly from one another (fig. 6). Some were swollen and had large dense basophilic nuclei; others had swollen, pale vesicular nuclei; all had deeply eosinophilic cytoplasm. The cell boundaries were poorly defined. A few cells were multinucleated. In one part of the endometrium, thicker than the rest, were masses of glands with large eosinophilic epithelial cells, often syncytial in appearance, with numerous round vesicular, somewhat enlarged nuclei. These glands were considered to be carcinomatous. In some of the glands neutrophils, red cells, monocytes, fibrin or protein precipitates were seen.

Near the internal os there was fairly deep hyalinization of the cervical tissues.

*Summary.*—The observations included: acute hemorrhagic necrotizing endometritis with necrosis of the adjacent myometrium in areas; chronic metritis (inner portion of the myometrium near the necrotic lining); radiation-induced changes in the epithelial cells of the endometrium and its glands and in the residual carcinomatous glands; chronic endometritis with old and recent hemorrhages; radiation-induced arterial changes and a radionecrotic plaque in the uterine wall at the level of the internal os; chronic cervicitis with cystic dilatation of the glands; bilateral chronic salpingo-oophoritis and perisalpingo-oophoritis; small tubo-ovarian abscess on the left side.

## SUMMARY OF THE OBSERVATIONS IN THE FOUR CASES IN WHICH THE UTERUS WAS SUBJECTED TO SPECIAL MICROSCOPIC EXAMINATION; RELATIONSHIP OF THE CHANGES TO RADIATION THERAPY

*Plaque at the Internal Os.*—In each of the 4 cases a plaque of necrotic tissue was found at the level of the internal os of the uterus. The plaque was definitely an effect of radiation. The structure and the genesis of the plaque will be discussed in detail farther on.

*Endometrium.*—In each case the endometrium, where present, was atrophic, often mildly edematous and at least focally infiltrated by chronic inflammatory cells, particularly near the plaque. In 3 cases (1, 2 and 4) the menopause had occurred years before radiation therapy was instituted. Hence atrophy of the endometrium might well have been the result of the cessation of ovarian function and cannot be definitely attributed directly to the irradiation of this tissue. In case 3 radiation may have played a role either directly by action on the endometrium or indirectly through its effect on the ovaries. These were definitely atrophic. The chronic endometritis observed in these cases was diffuse; it was associated with old hemorrhage in case 3, localized in the region of the plaque in cases 1 and 2 and present in the only strip of endometrium found in the uterine body in case 4. It is probable that the chronic endometritis was an effect, direct or indirect, of irradiation of the uterus, although the trauma resulting from the presence of radium applicator tubes in the uterus may have contributed. The slight separation of the connective tissue cells seen in the more superficial layers of the endometrium in these cases and considered indicative of edema is not uncommonly seen in the nonirradiated atrophic endometrium. Hence it cannot be proved to have

resulted from the irradiation. The diffuse necrosis of most of the endometrium in case 4 was probably the result of irradiation.

Squamous metaplasia of the columnar epithelium of the endometrium was observed in 1 case (3). No direct relation of this change to the radiation can be predicated. It is probable that the metaplasia was but a manifestation of the chronic endometritis, which was rather severe in this case.

*Myometrium.*—One definite fact stands out—in none of the 4 cases did the profound atrophy occur which had been described as an effect of radiation. In 2 cases (1 and 3) the uterus was distinctly larger than normal. The presence of a few small foci of leiomyoma could not have been responsible for the large size. In case 1 the muscle bundles of the myometrium seemed to be of normal size or even somewhat larger than normal (fig. 7). The only abnormality in the upper body were the arterial changes said to be characteristic of subinvolution of the uterus.[2]

In case 2 the muscle bundles in the inner half of the myometrium appeared somewhat atrophic, although the uterus as a whole was of about normal size. In this uterus, too, the arterial changes found in subinvolution of the uterus were in evidence.

In case 3, in which the uterus was large, there was a definite diffuse increase in connective tissue between the muscle bundles of the myometrium. The individual muscle fibers appeared enlarged (hypertrophy). It was difficult to determine accurately the size of the uterus in case 4 since it was received in several pieces. It was estimated to be of about normal size. The ectopic endometrium in the myometrium in this case (fig. 8) might have been partly responsible for its size.

It must be concluded that at least in these cases no diffuse muscular atrophy or diffuse hyalinization with contraction was produced by radiation despite the fact that full "cancer doses" were employed. It is possible that the uterus in each instance was so large before being irradiated that even a notable shrinkage produced by radiation still left it larger than normal or about normal in size. This possibility does not seem very plausible but cannot be absolutely invalidated. The deviations from the normal seen in the myometrium in 2 cases—atrophy of the inner portion of the myometrium in one and fibrosis and mild hypertrophy in the other—are too diverse to be considered effects of radiation.

The clumps of lymphocytes noted along the small veins and lymphatics in the myometrium, evidence of chronic metritis, were most numerous in those parts of the myometrium beneath or adjacent to the plaques. Their presence must have been the result of the inflammatory reaction in or near the plaques. Only a few of these clumps of lymphocytes were found in cases such as case 2, in which the inflammatory cellular response at the plaque was meager.

The focal acute metritis noted deep in the wall of the uterus in case 4 resulted from bacterial invasion of the plaque and so was an indirect result of irradiation.

Small foci of leiomyoma were found in the uterus in some of the cases. In most of them the neoplasm was only partly degenerated. Hyalinization, calcification and early cystic change were noted but were patchy in all. The muscle fibers in many seemed atrophic. These changes can be found in unirradiated leiomyoma and hence cannot be proved to have been produced by radiation. It is noteworthy that ectopic endometrium was present in the myometrium in 1 case in which the uterus was removed twelve years after the menopause. The ectopic endometrium showed the same degree of atrophy as the lining endometrium. As has been noted already, this atrophy could not be proved to be due to radiation.

Most of the changes in the arteries in the myometrium near the plaque can be considered effects of radiation and will be considered in the discussion of the structure and the genesis of the plaques later in this paper. In the middle layer of the myometrium in 2 cases (1 and 2) definite swelling of elastic tissue, the so-called vitreous change, was seen in the walls of arteries. Occasionally, apparently new small arteries were encased in wide expanses of altered degenerated elastic tissue, supposedly the remains of older larger arteries. These changes have been attributed to faulty involution of the uterus.[2] They are not radiation changes. The relation to radiation of hyalinization of small arteries in the depths of the endometrium or in the adjacent myometrium is more difficult to determine. In the 2 cases just mentioned (1 and 2), particularly in the first, many of these small arteries were hyalinized, but the endometrium was less damaged by radiation than in cases 3 and 4, and the larger arteries showed the changes accompanying subinvolution of the uterus. In case 3 hyalinization of similar small arteries was widespread, and the endometrium showed more radiation damage, but the arterial changes of uterine subinvolution were not present in the larger arteries. In case 4 the damage of the endometrium due to radiation was gravest, the arterial changes of subinvolution were not in

evidence in the large arteries of the middle layer and there were but few hyalinized arterioles near the endometrium. These observations seem to indicate that the hyalinization of the small arteries of the inner myometrium and the deeper portions of the endometrium may be a manifestation of uterine subinvolution or of radiation-induced change or possibly of some other condition and hence not a specific radiation change.

The peritoneum covering the uterus revealed no pathologic change directly attributable to radiation. The fibrous adhesions, the lymphocytic infiltration and the foreign body giant cell reaction to refractile foreign material in and on the serosa in case 1 could be the result of the old pelvic operation.

The marked chronic cervicitis noted particularly in cases 1 and 3 was probably indirectly the result of irradiation. The small ulcerated area on the vaginal aspect of the cervix in case 1 overlay an area of hyalinization of the fibromuscular coat in which radiation-induced vascular changes were present. There is little doubt that the changes in this part of the cervix were due to radiation.

The plasma cell infiltration deep in the wall of the cervix, particularly beneath the plaques, certainly was related, indirectly at least, to necrosis produced by radiation.

In short, the only uterine changes in the 4 cases which can be definitely considered effects of radiation are the plaque, the changes in the adjacent myometrium and the wall of the cervix, the arterial changes in the plaque and in the adjacent tissues, and the one area of extensive hyalinization of the cervix noted in case 1. The acute gangrenous endometritis and endocervicitis noted in case 4 and the chronic endometritis and cervicitis noted in the other cases are probably also the result of the irradiation though the modifying effects of trauma (introduction and removal of applicator tubes), hemorrhage and infection mask the more primary effects of radiation.

The most important effect of radiation on the uterus in these cases has not yet been discussed —the eradication of definite adenocarcinoma of the endometrium, demonstrated histologically before the therapy was begun. In each case a plaque of necrotic tissue was found at the level of the internal os. Only in case 2 was a second necrotic mass encountered. In this case the mass was polypoid and so necrotic that no vestiges, even wraithlike, of former structures, carcinomatous or not, could be identified. In no case were scars found in the myometrium. Only in case 2 were small calcified bodies encountered, possibly calcified cancer cells. In this case it is possible that the cancer had been present in the polypoid mass. In the other cases no probable site of the cancer was identified. At first it was thought that the necrotic plaques at the internal os were necrotic tumors, but later they were considered to be areas of radionecrosis. Of course the cancer might have been located at the level of the internal os and destroyed along with the normal structures at this site by the caustic action of the rays. It is also possible that the carcinoma was superficial, removed by curettage or completely destroyed by radiation. Endometrial reparative processes might then have hidden the site. However, in this paper photomicrographs of the cancer (figs. 1 and 2) reveal a histologic character different from that usually seen in the type strictly limited to the mucosa. From the histologic observations one would anticipate invasion of the myometrium. Yet no evidence of such infiltration was found in these 4 cases.

Finally, with the method of examination used in our cases it is unlikely that residual carcinoma was missed. To our knowledge, in previous reports on the changes in uteri excised after radiation therapy for carcinoma of the corpus uteri it was merely stated that there was no microscopic evidence of residual carcinoma (Healy and Brown [4]; Martin [5]) or that no special search had been made (Donovan and Warren [6]), or else microscopic observations, apparently on routine sections, were given without a statement of the details of the method of examination used (Arneson [7]; Corscaden [8]).

No definite indication of the mode of destruction of the cancer is afforded by the observations in the cases reported in this paper.

### IMPORTANT FEATURES OF THE RADIONECROTIC PLAQUES AT THE LEVEL OF THE INTERNAL OS

*Gross Features.*—In all the cases the plaque was an irregular mass of partly soft and partly firm material. The size was variable from case to case, and the surface was elevated above the adjacent portions of the endometrium, from which in some cases the plaque was well demarcated (case 3); in others, it was not (case 4). If no limits were apparent, the uterus was usually

4. Healy, W. P., and Brown, R. L.: Am. J. Obst. & Gynec. **38**:1, 1939.

5. Martin, C. L.: South. M. J. **33**:135, 1940.

6. Donovan, M. S., and Warren, S.: Surg., Gynec. & Obst. **74**:1106, 1942.

7. Arneson, A. N.: Am. J. Roentgenol. **36**:461, 1936.

8. Corscaden, J. A.: J. A. M. A. **126**:1134, 1944.

lined by necrotic material, and the plaque was but a particularly thick portion of the necrotic lining.

For the most part the color of the material in the plaque was some shade of brown or green. In case 6, in which the plaque could not have been present longer than one month, the earliest stage observed, the color was dark red. The hemorrhage which played a part in the production of the plaque and in the determination of its structure in all cases was probably responsible for the shade of red in the earlier stage and of brown in the later one. Microscopically, at the latter stage the plaque was composed, at least in part, of old hyalinized clot and hence was brownish. The green tints were probably the result of bacterial invasion and activity, since they were seen in the cases in which bacterial masses were found either in the plaque (case 4) or in the necrotic uterine lining (case 5).

The plaque not only projected into the uterine cavity (even as much as 6 mm.) but also extended into the adjacent myometrium (but no deeper than 5 mm.). The base of the plaque was usually well demarcated from the adjacent myometrium. In fact, in some of the cases the structure was easily dislodged.

In the cases in which the plaque was smaller (6 mm. in diameter) it was located in its entirety at the level of the internal os. In the cases in which it was larger (2.5 and 3.3 cm. in length) it extended up from the internal os into the body rather than down along the cervical canal and usually encircled the lumen.

*Microscopic Structure.*—Typically the plaque (fig. 3) was an infarct-like surface zone of complete necrosis. Beneath it typically lay a zone of partial hyalinization and edema and finally between this hyaline-edematous zone and the normal tissues a zone of edema and possibly of atrophy. The typical structure was seen best on the uterine body side of the internal os. Here the three zones could be readily defined and designated: the zone of complete necrosis, the zone of hyaline-edematous myometrium and the zone of edematous myometrium. The altered myometrium adjoining the necrotic surface zone was not merely a zone of reaction to the presence of necrotic tissue; it too showed the direct effect of the radiation.

In the typical necrotic surface zone there was a finely granular or faintly fibrillary pale material (case 1) or a more compact pale hyaline-like material (case 2). In it outlines of necrotic blood vessels and even faint tracings of muscle bundles could be identified. The exact size of the necrotic blood vessels in this zone and the presence or the absence of necrotic muscle bundles depended on the thickness of the necrotic zone, i. e., the depth of involvement of the myometrium or of the fibromuscular coat of the cervix. The endometrium was always destroyed. In some cases necrobiotic endometrial glands persisted near the free surface. Epithelium continuous with that on the surface of the adjacent endometrium or endocervix extended for variable distances up over the margins of the plaque in the typical case.

The hyaline-edematous zone and the edematous zone were often relatively thin (0.5 to 1 mm.). In the former, typically only nuclear remains of muscle cells persisted, with deposits of palely staining hyaline material around small blood vessels or even around the necrotic muscle cells themselves (fig. 4). In the latter case naked pyknotic nuclei set in small empty spaces were separated from adjacent nuclei by thin bands of hyaline material.

In the edematous zone the muscle fibers were separated by spaces and appeared thinner than normal. As the hyaline zone was approached, the nuclei of the muscle cells became more pyknotic, and the cytoplasm shrank and finally disappeared. The hyaline-edematous and edematous zones were not always sharply separated from each other. The hyaline-edematous zone, however, was usually well demarcated from the zone of complete necrosis. In case 2 two distinct zones were not found. In large areas practically normal myometrium adjoined the plaque.

In a typical case (case 1) over fairly large areas no cellular infiltration was noted in any zone with the exception of the completely necrotic zone, and here the infiltration was mild. Even here many structures which at first sight appeared to be neutrophils proved to be pyknotic nuclei of necrotic muscle cells. Over fairly wide areas there was also no cellular reaction in the more normal tissues bordering on the plaque. In areas, however, there was diffuse or focal cellular infiltration, at times massive, particularly in the hyaline-edematous or the edematous zone but sometimes in the lower part of the necrotic zone or in the normal tissues near the plaque The infiltration was best seen where hemorrhage or bacterial invasion had caused deviations from the typical structure of the plaque and of the adjacent zones of altered tissue.

In general, on the cervical side of the internal os the distinction between the hyaline-edematous zone and the zone of edema was not well defined. Often a hyaline zone much more compact than the hyaline-edematous myometrial zone was in

contact with the zone of complete necrosis on the surface.

In general there was no granulation tissue zone in contact with the necrotic tissue. In case 3, however, the complete disappearance of muscle elements and the persistence of connective tissue and blood vessels gave a definite impression of the presence of granulation tissue (fig. 4).

In the typical case bizarre giant fibroblasts or endothelial cells were present only in a few areas.

*Modifications in the Structure of the Necrotic Plaques Produced by Hemorrhage and Infection.*—The typical plaque was seen in 2 cases (1 and 2). It was modified in all the other cases and even in places in cases 1 and 2. The two predominant causes of modification were hemorrhage and bacterial invasion.

Masses of red cells were present in areas even deep in the typical plaque. It was not uncommon to find masses of fused red cells or of hyalinized dark red clot over all of the surface of the infarct-like portion of the plaque or on parts of it only. If the hemorrhage was more massive, the infarct-like zone might be completely replaced by altered hyalinized clot or at least by necrotic tissues so saturated with altered blood that they appeared to be hyalinized clot. This occurred in case 3, in which hyalinized clot rested directly on the hyaline-edematous myometrial zone (fig. 4).

Where clot rested directly on the hyaline zone there was a definite tendency for the plaque to be infiltrated by plasma cells, lymphocytes and monocytes in particular. Fewer neutrophils and eosinophils were seen. Red cells and hemosiderin-laden macrophages, as well as extracellular hemosiderin deposits of varying size, were often present.

Bacterial invasion was the other common cause of modification of the structure of the plaque. Where it was focal a nice contrast with the adjacent more typical plaque was afforded (cases 3 and 4). The entire invaded portion stained poorly and appeared partially autolyzed. Myriads of bacteria were present. Deep to this amorphous zone was a dense layer of neutrophils resting on a wide hyaline myometrial zone or a hyaline-edematous zone, rather heavily infiltrated by neutrophils. In this hyaline zone, which stained faintly, definite hyalinization of muscle fibers, sometimes with swelling, occurred. Hyalinized muscle cells were rarely seen except where bacterial invasion altered the plaque. The nuclei of these muscle cells faded gradually.

*Arterial Changes Associated with the Plaque.*—A variety of arterial changes were seen in and adjacent to the plaque. On the uterine body side the changes were most often in the hyaline-edematous or the edematous myometrial zone, next most commonly in the depths of the zone of necrosis and least often in the normal myometrium. On the cervical side the vascular changes were not only adjacent to the plaque but also at considerable distances away, deep in the fibromuscular coat (fig. 5).

The following arterial changes were observed: swelling of endothelial cells, with or without the subendothelial accumulation of fluid, fibrin, red cells, and a few inflammatory cells, particularly lymphocytes, monocytes and neutrophils; absence of endothelium; foam cell intimal plaques[3]; hyaline intimal thickening, even occlusive; hyaline necrosis of various portions of the arterial wall with or without dilatation, neutrophilic infiltration, hemorrhage and deposition of fibrin; more rarely fibroblastic intimal thickening and thrombosis with partial organization. Occasionally hemosiderin deposits in hyalin-thickened intima were seen. Some of these arterial changes are depicted in figure 5; others are illustrated in a previous article.[3] All of these in our opinion were due to radiation. Acute diffuse arteritis with necrosis, edema of the entire arterial wall with or without neutrophilic infiltration, and fibrinoid necrosis were observed most commonly near areas of bacterial invasion. Undoubtedly, this invasion played some part in their production, particularly where completely necrotic portions of the walls of arteries with massive neutrophilic infiltration were in contact with necrotic material containing masses of neutrophils and bacteria.

Hyalinization in varying degrees of severity was most common in arteries less than 300 microns in external diameter and was more common on the cervical side of the plaque than on the uterine body side. Hyalinization of arteries was present on the body side particularly when the plaque did not penetrate deeply into the myometrium (case 2).

## THE GENESIS OF THE PLAQUE

It is evident from the description of the properties of the plaque that it is essentially a zone of coagulation necrosis. The coagulation necrosis is identical with that found in many bland infarcts elsewhere in the body. The admixture of variable amounts of blood with the necrotic tissue is also usual in infarcts elsewhere. Swelling is a feature of such infarcts. It was found

in the uterine plaque, which in most of the cases projected somewhat into the uterine cavity. The swelling is probably due in both instances to the outpouring of protein-containing fluid and red cells into the necrotic area and subsequent coagulation. Like true infarcts, the typical radionecrotic plaque in the uterus is well demarcated from the adjacent viable tissues; in the latter in both instances marked changes in the walls and lumens of arteries are usually seen. When the uterine plaque becomes secondarily infected the changes simulate those of septic infarction.

There are, however, some apparent differences, notably, either the lack of a cellular inflammatory reaction in the areas of radionecrosis or in the surrounding viable tissues or at the most mild infiltration; the lack of granulation tissue in the typical case, and finally the long persistence of the radionecrotic tissues even when located on a surface. In addition, the diffuse hyalinization of the tissues bordering on the areas of radionecrosis, the obliteration of the capillary bed in the surrounding tissues and some features of the local arterial changes are not commonly observed in tissues adjacent to ordinary bland infarcts. However, if a comparison is made with old bland infarcts rather than with recent ones, the differences are not so striking. Around the periphery of an old splenic infarct, for example, it is not unusual to find at least a narrow band of partially hyalinized connective tissue with little cellular infiltration. However, diffuse hyalinization extending some distance from infarcts is not usual. Since in most of the cases described in this paper the uterine plaque was two and a half or more months old, a comparison with the older bland infarcts is probably appropriate.

The youngest plaque encountered in our material (case 6) was somewhat less than one month old. Numerous neutrophils were found in it. These may have been due to the infection present. In the endometrium adjacent to the plaque in this case small areas of necrosis limited to the most superficial layer of the endometrium were present (fig. 6). In the adjacent endometrial stroma edema, deposition of fibrin, fibrinoid necrosis of capillaries and of arterioles, dilatation of capillaries and small hemorrhages occurred. In the same region small clots, still retaining hemoglobin (orange-colored) or hyalinized (dehemoglobinized), were found just beneath the surface epithelium, portions of which were pushed outward into the uterine cavity by these deposits. The only definite evidence of necrosis here was the necrobiotic changes in the overlying surface epithelium. Perhaps such areas represent the early stage of plaque formation. The later desquamation of the epithelium with deposition of fibrin on the surface and among the subjacent connective tissue cells would simulate small infarcts.

Since the areas of radionecrosis are essentially areas of coagulation necrosis, they must result from the necrosis, in a relatively short interval of time, of all cells, epithelial, endothelial, fibroblastic and muscle, sufficiently close to the source of radiation, or from primary involvement of the blood vessels with occlusion and secondary infarction, or possibly from some change in the interstitial tissue or its fluids which interferes with the nutrition of the cells in the area. In favor of infarction is the observation that the seriously damaged portions of arteries are concentrated in the tissues close to the areas of necrosis. On the body side of the internal os in most of the cases these arterial changes were largely but not entirely confined to a layer of myometrium immediately adjacent to the dead tissue. All arteries passing through this fairly thin myometrial layer showed equivalent changes. Since these arteries ran parallel to one another, perpendicular to the lining of the uterine cavity, those who consider the areas of radionecrosis to be true infarcts must, in view of the close proximity of the occluded blood vessels to the area of necrosis, postulate coalescence of multiple small infarcts to explain the large plaque found in some of the cases. The problem of the exact mechanism of the production of areas of radionecrosis must remain unsettled until the relative importance of the various factors possibly concerned can be assessed. These include direct damage of individual cells, hyalinization of connective and of other tissues, vascular damage and possibly changes in the interstitial fluids. Even the origin of the various forms of hyalin encountered in the areas of radionecrosis and in the adjacent tissues is not clear. There are several possible sources: hyalinized blood clot, precipitated or gelled protein, old fibrin, hyalinized collagen and hyalinized cells.

The location of the plaque in the uterus and the relative roles of roentgen radiation and radium in its production remain to be discussed. In 1 case in which a well defined plaque was found, radium alone was used. It is reasonable to conclude that the radiations from radium produced the plaque in all the cases. The radium was inserted into the arms and the stem of a Y-shaped applicator so constructed that, once inserted, the arms could be spread apart to rest against the lateral wall of the uterine cavity

above the internal os. The stem remained in the cervical canal. It is highly probable that that part of the uterine wall at the level of the internal os was most closely applied to the source of radiation and that the effects of the latter were most intense there. The portions of the wall just above the internal os would receive a dose somewhat less but still quite intense. The shape of the mechanical applicator and the distribution of the radium in the arms and stem thus account for the location of the plaque. It is interesting that in a thick-walled organ such as the uterus a dose of radium sufficient to cause complete necrosis can be applied with little danger of serious damage, such as perforation. The main hazard is infection, which was present in some of the cases reported here. However all of the patients whose cases are reported here are still alive two years or more after irradiation of the uterus and hysterectomy.

## HISTORICAL SKETCH

Necrosis, slight or extensive, diffuse or focal, of the walls of the uterus subjected to radiation has been noted by numerous investigators. In the older literature Haendly [9] and Adler,[10] particularly the former, listed a variety of changes or gave detailed descriptions. Adler mentioned necrotizing inflammation of the uterus, diphtheritic endometritis, and "endometritis and metritis purulenta." He dealt mainly with cervical carcinoma. Haendly [9b] stated that there was slight necrosis of the superficial portion of the wall of the uterus in all cases in which the uterus was treated with radium or mesothorium but that it was not important. As more damaging effects of radiation he mentioned necrosis of the entire cervix and corpus in 1 case, gangrene of the uterus in 1, necrosis of the entire fundal portion of the uterus with sepsis, necrosis of the cervix and a portion of the corpus, and gangrene of the anterior or the posterior wall of the uterus in other cases. In 1 case "endometritis gangrenosa" and in 4 others "endometritis nekroticans" or "endometritis nekrotica septica" were diagnosed. He also laid great stress on hyaline degeneration, with or without necrosis, of the connective tissues and of smooth muscle in the muscular myometrium as well as in the walls of blood vessels. Many of Haendly's cases in which the patient came to grief were cases in which cancer of the cervix was treated with mesothorium or mesothorium and radium. The role of infection and of improper filtration or dosage in the production of the necrosis in his cases, perhaps, was not sufficiently well evaluated.

More recently Meyer [11] mentioned several layers in irradiated carcinoma of the corpus: a superficial zone of necrosis, a subjacent zone of necrobiosis and a deep infiltration or granulation tissue zone. Todd [12] noted radionecrosis of the whole lining of the uterus in a case in his series. Arneson,[7] in a case in his series, found the uterine cavity lined with a layer of hyalinized tissue, 3 to 4 mm. thick, with completely degenerated and unidentifiable cells in the deeper portions. Deep to this layer of necrosis the muscle cells showed hyalinization to a depth of several millimeters. Occasional thin-walled blood vessels were found along the line of demarcation between the superficial layer of necrosis and the underlying myometrium. He interpreted the presence of these blood vessels as a manifestation of an attempt at repair.

Localized areas of necrosis comparable to that described as a radionecrotic plaque in this paper have also been remarked on. Letulle [13] stated that radium causes deep cauterized lesions in uterine cancers. The scar in his case, corresponding to our radionecrotic plaque, was dry and hard and was characterized by long persistence, by long adherence to the normal tissues at the periphery, by prolonged inhibition of the usual inflammatory reaction and by fibrinoid necrosis of vessels which he felt was a most important sign of the causticity of radium.

Clark and Norris,[14] in summarizing their findings in numerous uteri subjected to radium, reiterated that there is no question but that radium exerts local action on the inner surface of the uterus, characterized at times by localized sloughing or necrotic areas involving the proximal layers of the myometrium, especially in those cases in which a moderately thorough curettage preceded the irradiation. In their cases the sloughs were at times as large as 5 cm. in length and 1 to 1.5 cm. in width but usually were smaller; they were located on either the anterior

9. Haendly, P.: (*a*) Arch. f. Gynäk. **109**:409, 1918; (*b*) Strahlentherapie **12**:1, 1921.

10. Adler, L.: Die Radiumbehandlung maligner Tumoren in der Gynäkologie, in Falta, W.; Gauss, C. J.; Meyer, H., und Werner, R.: Sonderbände zur Strahlentherapie, Berlin, Urban & Schwarzenberg, 1919, vol. 4.

11. Meyer, R.: Die pathologische Anatomie der Gebärmutter, in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1930, vol. 7, pt. 1, p. 477.

12. Todd, T. F.: Lancet **2**:821, 1938.

13. Letulle, M.: Presse méd. **30**:121, 1922.

14. Clark, J. G., and Norris, C. C.: Radium in Gynecology, Philadelphia, J. B. Lippincott Company, 1927, pp. 129-131.

or the posterior wall, usually the latter. The area of necrosis healed slowly, often persisting for weeks or months. Months after treatment macroscopic or histologic evidence of the action of radium might be absent. While localized fibrosis of the myometrium at the site of previous irradiation might be present, no massive cicatrices were encountered. Changes in blood vessels were quite localized to the site irradiated. These were generally moderate in degree and included thrombosis of the smaller uterine blood vessels.

Pullinger [15] classified such localized areas of radionecrosis in the uterus as infarcts and stated that they were examples of infarction at a surface. She claimed also to have seen rounded infarcts in the smooth muscle of the uterus. There was no detailed description of the necrotic patches. MacKee [16] considered similar areas of necrosis in the skin in third degree cutaneous roentgen ray reactions examples of dry gangrene. Corscaden [8] commented on the apparently localized effect of radium in the uterus. The sloughs and areas of cellular degeneration in his cases were limited in area and depth, giving him the impression that they corresponded to the location of the radium applicators. In a case in the series reported by Arneson and Hauptman [17] the necrosis and ulceration involved the cervical canal and the lower portion of the uterine cavity.

Vascular changes associated with areas of necrosis in the irradiated uterus have been stressed by Letulle,[13] Haendly [9b] and Pullinger.[15] In some of the other reports, previously cited, there is mention of vascular changes but no detailed report. Dyroff [18] noted vascular changes. Haendly [9b] observed capillary dilatation and stasis and changes in the walls of larger vessels. In the latter the end result of the changes was in his opinion a hyaline dead mantle, sometimes calcified, surrounding the thrombosed or obliterated lumen, even though at times the intima, at times the media or the adventitia, was most affected.

There has been considerable speculation on the genesis of localized areas of radionecrosis, whether in the uterus or elsewhere. Letulle [13] attributed the necrosis in his case to the causticity of radium without further explanation; Lacassagne,[19] in commenting on Letulle's case, attributed the necrosis to the action of $\beta$ rays and to the soft rays ($\gamma$ *mou*) of radium. He stated that the use of hard rays ($\gamma$ *dur*) would have prevented frank necrosis of normal tissues. Dobrovolskaia-Zavadskaia,[20] in her experimental work, described two zones concentrically placed around a source of radiation (radium and radon): an inner zone of necrosis and an outer zone of atrophy. The former she attributed to the action of soft rays ($\gamma$ *mou*); the latter, to hard rays ($\gamma$ *dur*). She believed that the centering of the zone of necrosis on the source of radiation ruled out vascular changes as the prime factor in the production of necrosis since the areas of necrosis did not correspond to the anatomic distribution of the local blood supply. Pullinger [15] considered the necrotic areas to be true infarcts and frankly attributed them to the vascular changes noted. She claimed that the following series of events occurred: hyperemia with stretching of the walls of capillaries and venules, resulting endothelial damage with exudation of serum and formation of small platelet thrombi, complete thrombosis of capillaries and venules, and even of larger vessels, leading to necrobiosis and anemic necrosis. In the uterus the thrombosis was followed by coagulation of the mucous membrane with subsequent sloughing. Deeper involvement of vessels led to gradual extension backward into the uterine wall. Healing then occurred by formation of granulation tissue and replacement of the necrotic areas by fibrous tissue, unless at the very beginning larger vessels were thrombosed. Then areas of quiet necrosis would persist. Her conclusions were challenged by Desjardins [21] on several grounds. Conclusions based on the use of full or tolerance doses, such as those used in the material examined by Pullinger, in his opinion could not furnish a satisfactory answer to the problem of the mechanism of cell death in irradiated tissues. The vascular changes could well be secondary to the direct destruction of cells and tissues by radiation rather than primary radiation changes resulting in cell death. If the vascular changes were primary, one must conclude that at least some of the cells constituting the tissues in the blood vessel walls were injured more readily by radiation than cells outside the vessels. According to his statement, doses affecting certain other cells do not affect endothelial cells; hence one could postulate that the changes in the latter were secondary to the destruction of the former cells. He mentioned in particular such radio-

15. Pullinger, B. D.: J. Path. & Bact. **35**:527, 1932.
16. MacKee, G. M.: Roentgen Ray Reactions and Injuries, in Pohle, E. A.: Clinical Roentgen Therapy, Philadelphia, Lea & Febiger, 1938, p. 743.
17. Arneson, A. N., and Hauptman, H.: J. A. M. A. **116**:29, 1941.
18. Dyroff, R.: Arch. f. Gynäk. **136**:141, 1929.
19. Lacassagne, A.: Presse méd. **30**:323, 1922.
20. Dobrovolskaia-Zavadskaia, N.: Lyon chir. **21**: 397, 1924.
21. Desjardins, A. U.: Am. J. Roentgenol. **28**:398, 1932.

sensitive cells as those of the seminal epithelium. That the exact genesis remains unsettled must be concluded from the foregoing discussion.

With regard to generalized atrophy of the myometrium, Haendly [9b] maintained that radiation caused two types of atrophy of smooth muscle, one characterized by deposition of hyalin and destruction of muscle nuclei; the other, by disappearance of the cytoplasm of the muscle cells with preservation of the nuclei. The latter appeared closely set. He claimed that the first type of atrophy was the more common in irradiated uteri and that because of it the uterus became a hard and shrunken senile organ. He was of the opinion that cessation of ovarian activity resulting from the use of radiation played little part; the shrinking of the uterus was due directly to radiation. In the cases now described no generalized atrophy of smooth muscle of either of these types was noted, despite the large doses of radiation used. The method of treatment—the total dose, the individual doses, the spacing of the doses and the filters used in our cases—might explain the difference in observed effects.

Finally, an interesting observation by Todd [12] should be mentioned. In 3 cases, in which the uterus was removed nine to nineteen months after a single dose of about 2,400 milligram hours, he found the normal endometrium replaced by a smooth shining membrane with telangiectatic zones and ruptured varices, the latter in 1 case situated just above the internal os. No information was given with regard to the microscopic observations.

SUMMARY

A thorough gross and microscopic study was made of 4 uteri excised after eradication of carcinoma of the endometrium by large doses of radium (about 6,000 milligram hours) and of roentgen radiation (about 4000 r in the midpelvis). Two other irradiated uteri were taken into consideration, although only routine sections of these were available. A carcinoma of the endometrium in one of these was destroyed by radiation.

The original site of the carcinoma in 5 of the 6 uteri could not be determined.

A localized plaquelike area of radionecrosis, essentially an area of coagulation necrosis, was found in the lining at or near the level of the internal os in 5 of the uteri. Changes were produced in the plaque by hemorrhage and infection. There is a question as to whether or not plaques of the type observed are true infarcts.

In the myometrial tissues adjacent to the uterine plaque two zones showing the effects of radiation were found: a superficial zone of hyalinization and edema with necrobiotic changes and a deeper zone of edema with atrophic changes. In the cervix a single zone of hyalinization was the usual finding. Vascular changes were encountered in these zones but were not confined to them.

Other observations included some degree of chronic endometritis, chronic cervicitis and endometrial atrophy; chronic metritis, mild and more or less focal, and other nonspecific lesions, including squamous metaplasia of the endometrial epithelium in 1 case.

# HEALING OF WOUNDS IN THE SKIN OF MICE PAINTED WITH 20-METHYLCHOLANTHRENE

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Benzpyrene applied to the epidermis of mice previous to the making of a wound in the skin stimulated the growth processes in the original as well as in the regenerating epithelium.[1] However, after prolonged painting with this substance, the ameboid movement of the proliferating epithelium into the defect was delayed or not increased at a rate commensurate with the intensification of the growth process, depending on duration of treatment. In order to determine whether this dissociation of growth and migration of the cells was due specifically to the action of benzpyrene or could be produced by other carcinogenic agents also, we have investigated the effect of 20-methylcholanthrene on the course of repair of the epidermis of mice treated with this carcinogenic substance previous to the making of the wound.

## MATERIAL AND METHODS

Sixty-four white Swiss mice, 2 to 3 months old at the beginning of the experiment, were used, being equally distributed as to litter mates and sexes whenever possible. The arrangement of the experiments was the same as in the previous investigation on the influence of benzpyrene with the exception that methylcholanthrene was substituted for the benzpyrene and that the carcinogen was applied by one stroke with a camel's hair brush (no. 6) instead of with a dropper.[2]

The animals were divided into three series: In the first series (16 mice) all the animals remained untreated as controls. In the second series (16 mice) the skin was painted with benzene three times weekly for two weeks, one month, two months or three months. In the third series (32 mice) the skin was painted with 20-methylcholanthrene dissolved in benzene in the concentration of 0.3 per cent. The carcinogen was applied for two weeks, one month, two months or three months.

At the end of the period of treatment the hair of those mice on which hair had grown in the interval was clipped a second time. An area of skin was marked with a round stencil measuring 3 mm. in diameter. This marked area was carefully excised with a pair of curved scissors, and regeneration was allowed to take place for three, five, eight or ten days. At the end of each experimental period, all mice were killed at the same time of the day, between 10 and 11 a. m., in order to avoid variations of the mitotic count due to the diurnal mitotic rhythm. A piece of skin including the wound was taken out and stretched on filter paper; the specimens were then fixed in 10 per cent formaldehyde solution and embedded in paraffin; the sections, 5 microns thick, were stained with hematoxylin and eosin.

The histologic observations are presented in tables 1 to 3. The ratio of basal to spinous cells is shown in column 4. The thickness of the epithelial layers was determined by counting the number of cell rows, and the size of the cells was considered in the various areas. The state of the original epithelium at a distance from the line of excision is seen in column 5; that of the epithelium at the margin of the wound, in column 6, and, lastly, that of the regenerating epithelial tongues advancing from the margin toward the center of the defect, in column 7. The length of the tongues is found in column 8. The mitotic count for the original epithelium (column 9) and that for the new epithelium (column 10) are given in multiples of the normal, the latter being in this strain 12 mitoses in 10,000 basal cells. As to further details of the method we refer to our former papers.

## OBSERVATIONS

*First Series* (control mice; untreated).—There was no difference in the progress of wound healing in the groups observed for two weeks, one, two or three months previous to excision. The following description is therefore concerned only with the different stages of regeneration and disregards the preceding period of observation.

(*a*) Old Epithelium: At a distance from the line of excision the epidermis at all stages was composed of two layers of epithelial cells, the upper consisting of spinous cells, the deeper of ellipsoid basal cells. The ratio of basal to spinous cells ranged between 2:1 and 2.5:1.

Near the line of excision the epithelium was thickened; three and five days after the making of the wound the epithelium was composed of four to six rows of cells instead of two as is normal. The spinous cells formed a thick layer of keratin. The basal cells were from 20 to 30 per cent larger than usual, cylindric in shape and oriented in a direction perpendicular to the surface. The nuclei were likewise enlarged; the structure of the nucleoli had become indefinite. Mitotic proliferation of the basal cells was intensified, showing a maximum of eight times the normal five days after operation (table 1, column 9). From eight to ten days after excision the number of cell rows was four to seven; the basal cells were now less hypertrophic, and after ten days a return to the resting state had occurred in

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These experiments were carried out in the Department of Pathology, New York University College of Medicine.

From the Laboratory of the Jewish Hospital and the Snodgras Laboratory of Pathology of the St. Louis City Hospital.

1. Silberberg, M., and Silberberg, R.: Am. J. Path. **20**:809, 1944.

2. Silberberg, M., and Silberberg, R.: Arch. Path. **38**:215, 1944.

some instances. Likewise, the mitotic count began to drop, but even after ten days it still remained elevated, the mean values varying from three and a half to one and a half times the normal after eight and ten days, respectively.

(*b*) New Epithelium: Enlarged epithelial cells migrated into the defect, growing out in a tonguelike fashion under the blood crust covering the defect. The advancing epithelial cells were spindle shaped, more so the closer they were to the tip of the tongue. The length of the tongues increased gradually from a mean of 0.29 mm. after three days to 0.87 mm. after ten days of observation (table 1, column 8). Only in 1 of 4 cases followed for ten days were the two tongues advancing from both margins of the wound just about ready to join. The thickness of the tongues increased during the first week from two rows of cells to four or five, after which it remained stationary or showed a decline to two to four rows after ten days (table 1, column 7).

Three days after excision there was scarcely any mitotic activity in the tongue, the mitotic count being about one fourth of that in normal resting epithelium. The number of mitoses, however, rose during the next two days and reached a peak of five times the normal (mean) five days after operation. Subsequently the mitotic count dropped gradually, but even ten days after excision the mean number of mitoses was still two and nine-tenths times higher than usual, the maximum being four and a half times and the minimum twice the normal (table 1, column 10).

*Second Series: Group 1* (benzene-treated mice; benzene applied for two weeks and for one month previous to the making of the wound).—(*a*) Old Epithelium: The epidermis at a distance from the wound consisted of two to three rows of epithelial cells, the ratio of basal to spinous cells having shifted in favor of the latter (table 2, columns 4 and 5). In the subcutaneous tissue there were scattered polymorphonuclear and mononuclear leukocytes, and here and there the epithelial cells showed a karyorrhexis, karyolysis or pyknosis of the nuclei. At the line of excision the epithelium was markedly thickened (four to nine cell rows), the maximum thickness of eight to nine rows being reached five days after operation (table 2, column 6). The basal cells of this epithelium were 10 to 20 per cent larger than the hypertrophic basal cells at the margin of the wound in untreated animals. Their mean mitotic count reached a peak of eight times the normal five days after excision (table 2, column 9). This compares with the maximum of six times (mean) the normal found in untreated controls. Subsequently the number of mitoses dropped sharply. After eight days the mean was only twice the normal, whereas in the control mice the number of mitoses ranged from twice to three and a half times the normal. Thus in the benzene-treated mice as compared with the controls there was an early intensification of mitotic proliferation, but the return to the resting state was somewhat more rapid than in the latter.

TABLE 1.—*Untreated Animals*

| Duration of Treatment | Days After Excision | Animals | Ratio of Basal to Spinous Cells | Rows of Cells in: Distant Epithelium | Rows of Cells in: Insertion of Tongue | Rows of Cells in: Tongue | Length of Tongue, Mm. | Mitosis in Multiples of the Normal: Mean, Maximum and Minimum Values: Original Epithelium | Mitosis in Multiples of the Normal: Mean, Maximum and Minimum Values: New Epithelium |
|---|---|---|---|---|---|---|---|---|---|
| 2 weeks | 3 | 323 | 2:1 | 2 | 4-5 | 2-3 | 0.31 | 5 max. 6½ min. 4 | ½ max. 1 min. 0 |
| | 5 | 235 | 2.3:1 | 2 | 5 | 3-4 | 0.51 | 5½ max. 6 min. 5 | 6 max. 6½ min. 5½ |
| | 8 | 239 | 2.3:1 | 2 | 5-6 | 3-4 | 0.62 | 3½ max. 5 min. 2½ | 4½ max. 5 min. 3 |
| | 10 | 243 | 2:1 | 2 | 4 | 4 | 1.01 | 1½ max. 2 min. 1 | 3 max. 4½ min. 2 |
| 1 month | 3 | 247 | 2:1 | 2 | 4-5 | 2 | 0.29 | 2½ max. 3 min. 2 | 0 |
| | 5 | 251 | 2.5:1 | 2 | 5-6 | 2-3 | 0.52 | 4 max. 6 min. 3 | 4 max. 6 min. 3 |
| | 8 | 255 | 2:1 | 2 | 4-5 | 4-5 | 0.66 | 2 max. 3 min. 1 | 2½ max. 3½ min. 1½ |
| | 10 | 259 | 2.5:1 | 2 | 4-5 | 2-3 | Closing | 2 max. 2 min. 2 | 3½ max. 4 min. 3 |
| 2 months | 3 | 263 | 2.5:1 | 2 | 4-5 | 2-3 | 0.27 | 5½ max. 7 min. 4 | ¼ max. ½ min. 0 |
| | 5 | 267 | 2.2:1 | 2 | 5-6 | 2-3 | 0.42 | 4¼ max. 5 min. 3 | 5 max. 6 min. 4 |
| | 8 | 271 | 2.5:1 | 2 | 5-6 | 3-4 | 0.58 | 2 max. 3 min. 1½ | 4 max. 5 min. 3½ |
| | 10 | 275 | 2.2:1 | 2 | 5-6 | 2-3 | 0.88 | 1½ max. 2 min. 1 | 2½ max. 3 min. 2 |
| 3 months | 3 | 279 | 2:1 | 2 | 5-6 | 3-4 | 0.29 | 3½ max. 5 min. 2 | ½ max. 1 min. 0 |
| | 5 | 283 | 2.2:1 | 2 | 5-6 | 3-4 | 0.39 | 6 max. 8 min. 5 | 5 max. 6½ min. 3½ |
| | 8 | 287 | 2:1 | 2 | 5-6 | 3-4 | 0.82 | 2 max. 2½ min. 1 | 4 max. 5 min. 3 |
| | 10 | 291 | 2.2:1 | 2 | 6-7 | 4 | 0.72 | 3½ max. 4 min. 3 | 2½ max. 3 min. 2* |
| (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) | (10) |

* Papilloma noted after five weeks of painting.

(*b*) New Epithelium: In the benzene-treated mice the epithelial tongues advanced more rapidly toward the center of the wound than those in the untreated mice (table 2, column 8). Three days after operation the mean length of the tongues was 0.39 mm. in the 2 benzene-treated mice, whereas in the 4 untreated mice it was only 0.29 mm. Five days after excision the corresponding mean figures were 0.57 mm. in the benzene-treated mice and 0.46 mm. in the 4 untreated animals. After eight days the tongue was 0.68 mm. long in one of the benzene-treated mice, while in the other both tongues had already met in the center, indicating complete epithelization of the defect. In none of the untreated animals had the tongues met at this stage, the mean length of the tongues being 0.67 mm. Ten days normal in the benzene-treated animals, compared with two and a half to four and a half times the normal in the untreated mice (table 2, column 10). Thus, also in the regenerating epithelium the return to the resting state was accelerated in the benzene-treated mice.

*Second Series: Group 2* (benzene applied for two and for three months previous to the making of the wound). —(*a*) Old Epithelium: The structure of the epidermis at some distance from the wound as well as near the line of excision did not differ significantly from that seen after application of benzene for shorter periods. The number of mitoses rose and fell about as steeply as after two weeks or one month of treatment, or perhaps even somewhat more steeply inasmuch as the peaks were already present after three days and the drop was

TABLE 2.—*Benzene-Treated Animals*

| Duration of Treatment | Days After Excision | Animals | Ratio of Basal to Spinous Cells | Rows of Cells in: Distant Epithelium | Rows of Cells in: Insertion of Tongue | Rows of Cells in: Tongue | Length of Tongue, Mm. | Mitosis in Multiples of the Normal: Mean, Maximum and Minimum Values: Original Epithelium | Mitosis in Multiples of the Normal: Mean, Maximum and Minimum Values: New Epithelium |
|---|---|---|---|---|---|---|---|---|---|
| 2 weeks | 3 | 232 | 1:1 | 3 | 4-5 | 2-3 | 0.39 | 5 max. 6 min. 4 | 5 max. 6 min. 4 |
| | 5 | 236 | 1:1 | 3 | 8-9 | 4-5 | 0.56 | 8 max. 10 min. 6 | 5 max. 6 min. 5 |
| | 8 | 240 | 1:0.75 | 3 | 7 | 3-4 | 0.68 | 2 max. 2 min. 2 | 2 max. 2 min. 2 |
| | 10 | 244 | 1:1 | 3 | 7-8 | 3-4 | Closing | 1½ max. 3 min. 1 | 2 max. 2½ min. 1½ |
| 1 month | 3 | 248 | 1:1 | 3 | 6-7 | 3-4 | 0.39 | 5 max. 6 min. 4 | 3½ max. 4 min. 2½ |
| | 5 | 325 | 1:1.5 | 2-3 | 5-6 | 2-3 | 0.58 | 6 max. 7 min. 4½ | 7 max. 7½ min. 6 |
| | 8 | 326 | 1.5:1 | 3 | 6-7 | 3-4 | Closing | 1½ max. 2 min. 1 | 3½ max. 4 min. 3 |
| | 10 | 327 | 1.2:1 | 2-3 | 5-6 | ... | Closed | 1 max. 1½ min. 1 | 2 max. 2½ min. 1½ |
| 2 months | 3 | 264 | 1.1:1 | 3 | 6-7 | 4-5 | 0.37 | 7 max. 10 min. 6 | 5½ max. 6½ min. 4 |
| | 5 | 268 | 1.2:1 | 3-4 | 6-7 | 3-4 | 0.72 | 4 max. 5 min. 3 | 4½ max. 5½ min. 4 |
| | 8 | 272 | 1:1.2 | 3 | 6-7 | 3-4 | 0.63 | 3 max. 4 min. 2½ | 2 max. 2½ min. 1½ |
| | 10 | 276 | 1.5:1 | 2-3 | 6-7 | ... | Closed | 2 max. 3 min. 1½ | 1 max. 1½ min. 1 |
| 3 months | 3 | 280 | 1:1 | 3 | 6-7 | 4 | 0.37 | 5 max. 6 min. 3 | 5 max. 6 min. 3½ |
| | 5 | 284 | 1:1 | 3-4 | 7-8 | 4-5 | 0.67 | 3 max. 4 min. 2 | 4½ max. 5 min. 4 |
| | 8 | 288 | 1:1.2 | 3 | 6-7 | ... | Closing | 1½ max. 2 min. 1 | 2 max. 3 min. 1 |
| | 10 | 292 | 1:1 | 3 | 4-5 | ... | Closed | 1½ max. 3 min. 1 | 2 max. 3 min. 1 |
| (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) | (10) |

after operation the wounds were closing or closed in the benzene-treated mice, whereas in only 1 of the 4 untreated animals had the tongues approached each other closely. The thickness of the epithelial tongues resembled that found in the untreated controls. As in the latter, the tongues were composed of from two to five rows of cells (table 2, column 7).

The number of mitoses in the outgrowing epithelium was markedly increased over that found in the untreated controls. Three days after excision the mean number of mitoses was three and a half to five times the normal, whereas it was below normal in the untreated mice. After five days the mean mitotic count was five to seven times the normal, compared with four to six times in the untreated mice. After eight and ten days the mean of the number of mitoses had dropped to twice the noticeable as early as five days after operation (table 2, column 9).

(*b*) New Epithelium: The growth processes were similar to those seen after two weeks or one month of painting with benzene; no further intensification had taken place after two or three months' application of the substance. The lengths of the tongues as well as the numbers of mitoses were similar to those found after treatment with benzene for shorter periods (table 2, columns 8 and 10).

*Third Series: Group 1* (methylcholanthrene-treated mice; methylcholanthrene applied for two weeks and for one month previous to the making of the wound).—(*a*) Old Epithelium: The epidermis at a distance from the defect was markedly thickened and keratinized. Pearls of keratin filled the hair follicles, and frequently

the hair had fallen out. Here and there the nuclei of the epithelial cells had undergone pyknosis, karyolysis and karyorrhexis. Foci of polymorphonuclear and mononuclear leukocytes were found in the epidermis and in the subcutaneous tissue. In the latter, there was edema, and the collagenous fibers were fragmented. The epidermis was composed of three to five rows of cells, compared with two to three in mice painted with benzene alone (tables 2 and 3, column 5). The ratio of basal to spinous cells had shifted markedly in favor of the latter (table 3, column 4).

At the margin of the wound the thickness of the epidermis varied from four to ten rows during the period of observation. It was thus somewhat thicker than in the mice painted with benzene, which showed during the corresponding period variations of from four to nine rows of cells (tables 2 and 3, column 6). The basal cells were as hypertrophic as after treatment with benzene alone. Likewise, after two weeks of treatment with methylcholanthrene, the rise of the mitotic count was of the same order as in the benzene-painted animals (tables 2 and 3, column 9). However, after one month of application of methylcholanthrene the return to the resting state during the later stages of wound healing was delayed as compared with the course of events in both the normal and the benzene-painted mice. Ten days after excision, the mean of the mitotic counts still remained four to six times higher than normal, whereas in the benzene-treated and the untreated control mice the number of mitoses had dropped to almost normal (tables 1 to 3, column 9).

(*b*) New Epithelium: Three days after operation the epithelial tongues advancing into the defect (table 3, column 8) were 0.40 mm. (mean) long in the mice painted for two weeks; they were only 0.345 mm. (mean) long in the mice painted for one month. Five days after excision the values were 0.52 mm. (mean) in the animals painted for two weeks and 0.345 mm. (mean) in those painted for one month. Eight and ten days after the making of the wound the defects were epithelized to a similar extent in both the benzene-painted and the methylcholanthrene-painted group.

The tongues consisted of two to six rows of cells as compared with two to five rows in the benzene-treated mice. The regenerating epithelial cells were almost twice as large as resting epidermal cells. After application of the carcinogen for two weeks the mean values for the mitotic counts (table 3, column 10) were slightly higher in the methylcholanthrene series. This difference became more apparent after application of the carcinogen for one month. Moreover, whereas after painting with benzene the mean mitotic count had dropped to twice the normal ten days after excision, the mitotic count was four or five and a half times the normal in the mice that had been painted with methylcholanthrene.

*Third Series: Group 2* (methylcholanthrene applied for two and for three months previous to the making of the wound).—(*a*) Old Epithelium: The epidermis at a distance from the wound had undergone further thickening, the average thickness being five to six rows three and five days, and four to five rows eight and ten days, after excision. Large amounts of keratin had been laid down. The spinous cells outnumbered the basal cells now as much as 2.5:1 in 2 animals (table 3, columns 4 and 5). Regression of the epithelial cells was more marked, and infiltration by polymorphonuclear and mononuclear leukocytes was more widespread than previously. However, the hypertrophy of the epidermal cells was the same as that found after two weeks or one month of painting.

The thickness of the marginal epithelium varied during the period of observation from five to ten cell rows, averaging seven to eight rows throughout the ten days of observation. The number of mitoses was greatly increased, particularly after three months of painting, when the mean values reached a maximum of from nine and a half to eleven and a half times the normal (table 3, column 9). The mitotic count was independent of the stage of repair, remaining high throughout the period of observation.

(*b*) New Epithelium: The regenerating epithelial tongues were definitely shorter in the mice painted with methylcholanthrene than in the benzene-painted and the untreated animals (tables 1 to 3, column 8). After two months of application of methylcholanthrene there was little outgrowth during the first three days following operation (mean, 0.075 mm.). But this inhibition was only temporary, and at later stages the advancement of the tongues was faster. However, after three months of painting the tongues did not increase much in length during the time of observation, being 0.295 (mean) mm. long three days after excision and 0.35 mm. (mean) after ten days.

The thickness of these short tongues showed marked variations of from two to seven rows of cells (table 3, column 7). The degree of hypertrophy of the advancing epithelial cells was similar to that found after shorter periods of painting. The mitotic proliferation had increased to a mean maximum of eight and three-fourth times the normal (table 3, column 10). After two months of treatment the wounds closed ten days after excision. There was a maximum of mitotic activity after five days, followed by a slow drop during the next three days and a somewhat steeper decline during the last days of observation, at the time when the wounds closed. However, there was no return to the normal resting state, and in accordance with the increased number of mitoses in the original epithelium, the mitotic count remained three and a fourth times the normal (table 3, column 10). After three months of painting the mean mitotic count was seven times the normal three days subsequent to excision, and it remained high (six and a fourth to eight and a half times the normal) throughout the period of observation.

## COMMENT

Repair in the skin of unpainted mice followed the course observed in previous investigations:[1] Wounds 3 mm. in diameter at the time of excision were not completely closed after ten days. There was a distinct mitotic cycle typical of regenerating epithelium as shown by Loeb and coworkers.[3] In the original epithelium the mitoses increased at first more rapidly than those in the new regenerating epithelium. In both maximum was reached at five days, and then the mitotic count declined; but even after ten days the number of mitoses in the old epithelium was twice as high and the number in the regenerating epithelium three times as high as in the resting skin.

3. Loeb, L.: Arch. f. Entwcklngsmechn. d. Organ. **24**:638, 1907. Loeb, L., and Addison, W. H. F.: ibid. **32**:44, 1911. Loeb, L., and Spain, K. C.: J. Exper. Med. **21**:193, 1915.

TABLE 3.—*Methylcholanthrene-Treated Animals*

| Duration of Treatment | Days After Excision | Animals | Ratio of Basal to Spinous Cells | Rows of Cells in | | | Length of Tongue, Mm. | Mitosis in Multiples of the Normal: Mean, Maximum and Minimum Values | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Distant Epithelium | Insertion of Tongue | Tongue | | Original Epithelium | New Epithelium |
| 2 weeks | 3 | 233 | 1.4:1 | 4-5 | 7 | 4 | 0.37 | 5 max. 6<br>min. 4 | 5 max. 5½<br>min. 4 |
| | 3 | 234 | 1.1:1 | 5 | 7-8 | 4-5 | 0.43 | 5½ max. 6<br>min. 4½ | 5 max. 5½<br>min. 4½ |
| | | | | | | | Mean 0.40 | Mean 5¼ | Mean 5 |
| | 5 | 237 | 1:1 | 4 | 7-8 | 4 | 0.51 | 7 max. 9<br>min. 5½ | 6½ max. 8<br>min. 5 |
| | 5 | 238 | 1:1.2 | 4-5 | 8-9 | 4-5 | 0.53 | 6 max. 7<br>min. 5½ | 7 max. 8<br>min. 6½ |
| | | | | | | | Mean 0.52 | Mean 6½ | Mean 6¾ |
| | 8 | 241 | 1:1.2 | 3-4 | 6-7 | 4-5 | 0.83 | 2½ max. 3<br>min. 2 | 3½ max. 4<br>min. 2½ |
| | 8 | 242 | 1:1.3 | 5 | 8-9 | 5-6 | 1.05 | 4 max. 5½<br>min. 3½ | 5 max. 5½<br>min. 4½ |
| | | | | | | | Mean 0.94 | Mean 3¼ | Mean 4¼ |
| | 10 | 245 | 2:1 | 3 | 7-8 | 3-4 | Closing | 1½ max. 2<br>min. 1 | 1 max. 1½<br>min. 1 |
| | 10 | 246 | 1.5:1 | 3 | 6 | 4 | Closed | 1½ max. 1½<br>min. 1½<br>Mean 1½ | 1½ max. 2<br>min. 1<br>Mean 1¼ |
| 1 month | 3 | 231 | 1:1 | 5 | 5-6 | 2-3 | 0.39 | 5 max. 6<br>min. 4½ | 2½ max. 3<br>min. 2 |
| | 3 | 249 | 1:1.25 | 3-4 | 6-7 | 3-4 | 0.30 | 4 max. 5½<br>min. 2½ | 2 max. 3<br>min. 1 |
| | | | | | | | Mean 0.345 | Mean 4½ | Mean 2¼ |
| | 5 | 253 | 1:1.5 | 4-5 | 9-10 | 4-5 | 0.38 | 5 max. 6½<br>min. 3½ | 7 max. 9<br>min. 5 |
| | 5 | 254 | 1:1.3 | 4-5 | 8-9 | 3-4 | 0.31 | 6 max. 6½<br>min. 4½ | 6½ max. 9<br>min. 5 |
| | | | | | | | Mean 0.345 | Mean 5½ | Mean 6¾ |
| | 8 | 257 | 1:1.1 | 3 | 4-5 | ... | Closing | 7 max. 9<br>min. 5½ | 4½ max. 5<br>min. 3 |
| | 8 | 258 | 1:1.2 | 3-4 | 6-7 | ... | Closing | 5½ max. 6<br>min. 3½<br>Mean 6¼ | 2½ max. 3<br>min. 2<br>Mean 3½ |
| | 10 | 261 | 1:1.25 | 4-5 | 5-6 | 3 | 0.69 | 6 max. 6<br>min. 6 | 5½ max. 6<br>min. 5 |
| | 10 | 262 | 1:1.1 | 3-4 | 4-5 | ... | Closed | 4 max. 5½<br>min. 3½<br>Mean 5 | 4 max. 5<br>min. 3½<br>Mean 4¾ |
| 2 months | 3 | 265 ‡ | 1:1.2 | 4-5 | 5-6 | 2-3 | 0.08 | 6½ max. 8½<br>min. 5½ | 2 max. 2½<br>min. 1⅛ |
| | 3 | 266 ‡ | 1:1 | 4-5 | 6 | 1-2 | 0.07 | 8 max. 10<br>min. 6 | 3½ max. 4<br>min. 3 |
| | | | | | | | Mean 0.075 | Mean 7¼ | Mean 2¾ |
| | 5 | 269 † | 1:1.5 | 5-6 | 7-8 | 2-3 | 0.57 | 6 max. 7<br>min. 5 | 7½ max. 9<br>min. 5 |
| | 5 | 270 | 1:2 | 6-7 | 7-8 | 1-2 | 0.41 | 10 max. 12<br>min. 8 | 10 max. 12<br>min. 8 |
| | | | | | | | Mean 0.49 | Mean 8 | Mean 8¾ |
| | 8 | 273 | 1:2 | 3 | 5-6 | 4-5 | 0.69 | 5½ max. 6½<br>min. 4 | 5 max. 6<br>min. 4½ |
| | 8 | 274 | 1:1.25 | 3-4 | 8-9 | 4-5 | 0.87 | 10 max. 12<br>min. 9½ | 8½ max. 9<br>min. 7 |
| | | | | | | | Mean 0.78 | Mean 7⅞ | Mean 6¾ |
| | 10 | 277 | 1:1.2 | 4-5 | 7-8 | ... | Closing | 8 max. 10<br>min. 7 | 3½ max. 4<br>min. 3 |
| | 10 | 278 | 1:1.5 | 4-5 | 8 | ... | Closed | 10 max. 11<br>min. 7<br>Mean 9 | 3 max. 4<br>min. 2½<br>Mean 3¼ |
| 3 months | 3 | 281 ‡# | 1:1.5 | 5-6 | 8-9 | 3-4 | 0.28 | 12 max. 15<br>min. 10 | 6 max. 8<br>min. 5½ |
| | 3 | 282 ‡ | 1:1.8 | 5-6 | 8-9 | 2-3 | 0.31 | 11 max. 13<br>min. 10 | 8 max. 9<br>min. 7 |
| | | | | | | | Mean 0.295 | Mean 11½ | Mean 7 |
| | 5 | 250 ‡ | 1:1.5 | 5-6 | 6-7 | 4-5 | 0.42 | 7 max. 9<br>min. 6 | 5½ max. 6½<br>min. 4½ |
| | 5 | 286 * | 1:2.5 | 5-6 | 7-8 | 3-4 | 0.32 | 12 max. 14<br>min. 10 | 7 max. 8<br>min. 6 |
| | | | | | | | Mean 0.37 | Mean 9½ | Mean 6¼ |
| | 8 | 289 § | 1:2.2 | 5 | 7-8 | 3-4 | 0.29 | 12 max. 14<br>min. 11 | 7½ max. 8<br>min. 6½ |
| | 8 | 290 ‡ | 1:2.5 | 5-6 | 6-7 | 4 | 0.39 | 10 max. 12<br>min. 9 | 6½ max. 7½<br>min. 5 |
| | | | | | | | Mean 0.34 | Mean 11 | Mean 7 |
| | 10 | 293 ‡ | 1:2.2 | 7-8 | 9-10 | 6-7 | 0.29 | 8 max. 10<br>min. 7 | 8 max. 10<br>min. 7½ |
| | 10 | 294 ‡ | 1:2 | 5-6 | 7-8 | 5-6 | 0.41 | 14 max. 15<br>min. 12 | 9 max. 10<br>min. 8 |
| | | | | | | | Mean 0.35 | Mean 11 | Mean 8½ |
| (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) | (10) |

* Papilloma noted after five weeks of painting.
† Papilloma noted after six weeks of painting.
‡ Papilloma noted after eight to nine weeks of painting.
§ Papilloma noted after twelve weeks of painting.
\# Papilloma developed at the line of excision.

Our previous observations concerning the effect on the repair of wounds of benzene applied to the skin before operation could be confirmed. Skin thus treated was thicker and reacted to the stimulation exerted by the wound more rapidly and more intensely than did normal skin. The mitotic count rose more rapidly and to a higher level than that in unpainted animals, and the migrating of the epithelial cells into the defect

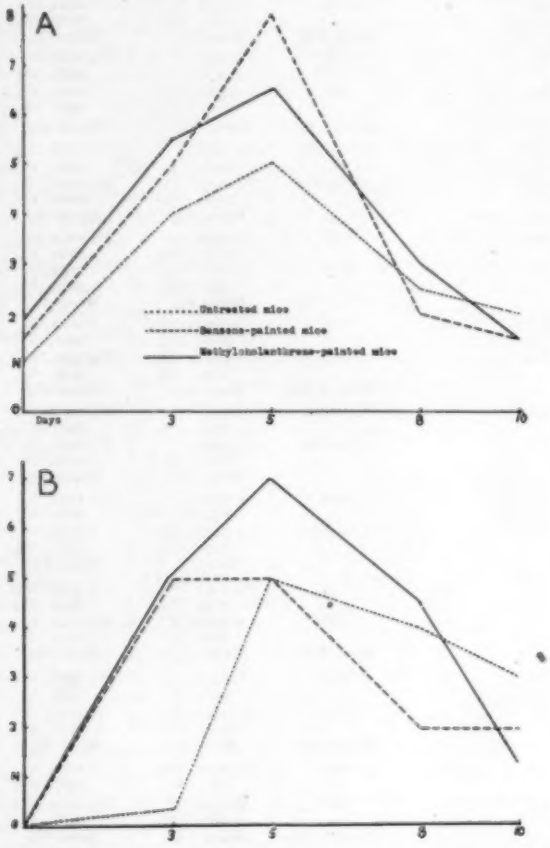


Chart 1.—*A*, mitotic cycle in the original epithelium during ten days of wound repair in control mice and in animals treated for two weeks. *B*, mitotic cycle in the new regenerating epithelium. *N* stands for the normal value and the numbers above *N* for multiples of the normal value.

was accelerated over the normal. Consequently, epithelization of the wounds was hastened, and the drop of the mitotic activity, approximately coinciding with the closing of the wound (Loeb [4a]), was more rapid and reached a lower level in treated than in untreated mice. This stimulating effect of benzene was limited, however, and could not be intensified by prolonged application.

Methylcholanthrene dissolved in benzene and applied to the epidermis previous to excision affected epithelization of the wounds to a varying degree depending on the duration of treatment previous to the making of the wound. After two weeks of painting, epithelization was accelerated over the normal, and the defects were closed after ten days of observation. This effect on epithelization was comparable to that exerted by benzene. The mitotic activity in the original epithelium of the methylcholanthrene-treated mice was increased in comparison with that observed in unpainted animals. In the new epithelium mitotic activity was likewise intensified as compared with that in either untreated or benzene-painted mice.

Chart 1 demonstrates the mitotic activity in the old and the new epithelium in normal mice and in animals painted with benzene and methylcholanthrene for two weeks. In all three groups the typical mitotic cycle existed in the old as well as in the new epithelium: There was an early rise of the mitotic activity, then a drop to normal or nearly normal.

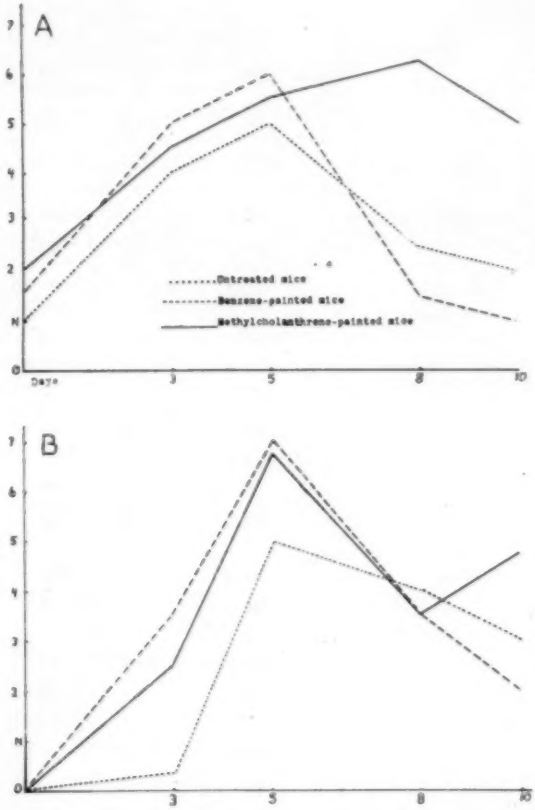


Chart 2.—*A*, mitotic cycle in the original epithelium during ten days of wound repair in control mice and in animals treated for one month. *B*, mitotic cycle in the new regenerating epithelium. *N* stands for the normal value and the numbers above *N* for multiples of the normal value.

After one month of treatment with methylcholanthrene (chart 2) a change in the mitotic

4. (*a*) Loeb, L.: J. M. Research **41**:247, 1919. (*b*) Akaiwa, H.: ibid. **40**:311 and 371, 1919.

cycle was noted in the old epithelium. While the absolute number of mitoses did not exceed that seen after two weeks of painting, there was no marked decline in the proliferative activity ten days after excision. This deviation from the ordinary cycle was especially striking in 3 of 4 mice in which the epithelial tongues had met and closed the defect, an occurrence which under normal conditions coincides with a steep drop of the number of mitoses. In the new epithelium, on the other hand, the mitotic cycle was still present, the sharp drop between the fifth and the eighth day being due possibly to some unexplained accidental factors.

In the mice painted for two months with methylcholanthrene (chart 3), the disturbance of the cycle of mitotic proliferation as shown in the lack of decline in the mitotic activity in the old epithelium during the second part of the cycle became more conspicuous. However, the migration of the epidermal cells into the wound was extremely slow during the first three days. This lag of cell movement was even more striking since the number of mitoses was higher than after two weeks or one month of painting. Closure of the wound occurred after ten days of observation, at the same time as after two weeks of treatment. In view of the intense mitotic activity present after two months of painting, the epithelization of the defect was comparatively delayed, indicating beginning dissociation of cell proliferation and cell migration. The mitotic proliferation in the new epithelium continued to show the typical cycle: an early rise which was greater than that observed in controls or in benzene-treated mice, followed by a decline during the later stages of wound healing.

After three months of treatment with methylcholanthrene previous to excision (chart 4) the mitotic activity no longer revealed a cyclic character: The mitotic count in the old epithelium was high at the beginning and remained high throughout the period of observation; migration of the epithelium, on the other hand, made scarcely any progress after the first three days. The dissociation of cell movement and cell proliferation was now pronounced. Besides, there was a marked change in the mode of reaction of the new epithelium: Whereas, after shorter periods of painting the regenerating epithelium exhibited a rhythmic cycle during the course of repair, it failed to do so after three months of treatment. This seemed even more remarkable since the newly formed epithelium was at no time under the direct effect of the carcinogenic substance. This lack of a cyclic decline may perhaps be explained partly by the failure of the wounds to close, but probably it is largely due to the effect of methylcholanthrene.

A comparison of the course of healing of wounds subsequent to painting with benzpyrene and with methylcholanthrene revealed the following facts: At any given stage the keratinization of the epithelium was more marked under the influence of benzpyrene than under that of methylcholanthrene; regressive changes and inflammatory reaction in the epidermis and in the connective tissue were likewise more pronounced in the benzpyrene series. After two weeks of painting with benzpyrene, the stimulation of proliferation

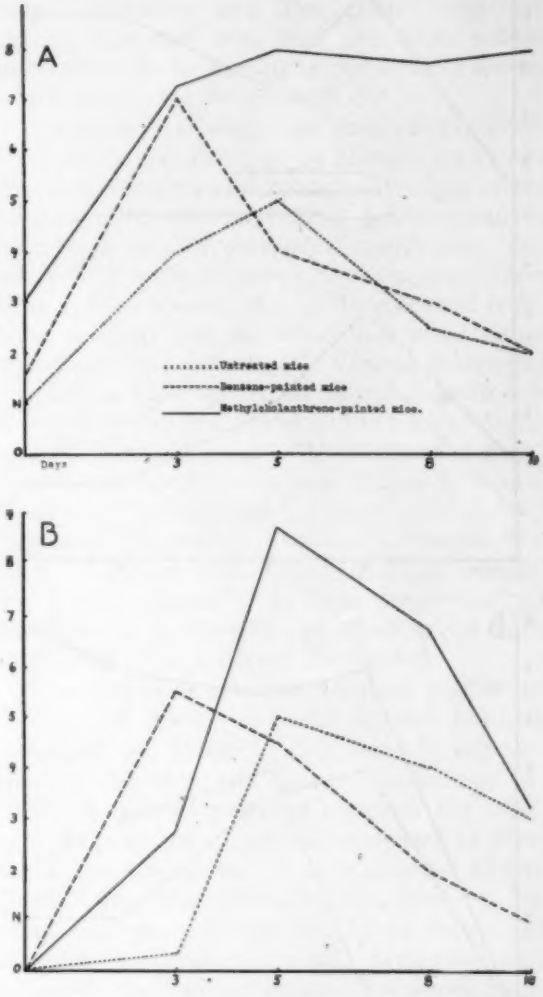


Chart 3.—*A*, mitotic cycle in the original epithelium during ten days of wound repair in control mice and in animals treated for two months. *B*, mitotic cycle in the new regenerating epithelium. *N* stands for the normal value and the numbers above *N* for multiples of the normal value.

was somewhat more intense, and the mitotic counts rose slightly more rapidly than after the application of methylcholanthrene. On the other hand, the rhythmic cycle of epithelial proliferation in the mice painted with methylcholanthrene was more similar to that seen in the un-

treated series than to that in the benzpyrene group. After one month of treatment the intensification of proliferation and the cycle of mitotic activity took a similar course in both experimental groups. After two months of painting there was marked inhibition of the migration of epithelial cells into the wounds in the benzpyrene group, whereas in the methylcholanthrene series the inhibition of cell movement was relative rather than absolute. In the latter group the progress of epithelization was not commensurate with the increased rate of cell proliferation. After three months of treatment, however, the inhibition of cell migration was marked in both the benzpyrene-treated and the methylcholanthrene-treated animals.

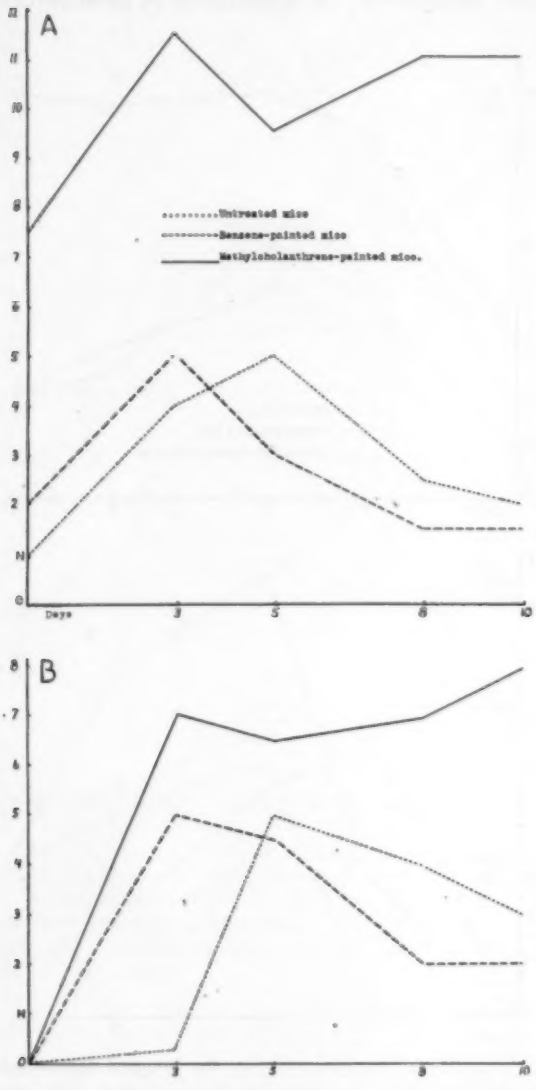


Chart 4.—*A*, mitotic cycle in the original epithelium during ten days of wound repair in control mice and in animals treated for three months. *B*, mitotic cycle in the new regenerating epithelium. *N* stands for the normal value and the numbers above *N* for multiples of the normal value.

The effect of methylcholanthrene on regenerating epithelium may thus be considered as more graded than that exerted by benzpyrene. This effect may be correlated with the observations of Nicod and Regamey,[5] who found that methylcholanthrene produced fewer carcinomas than benzpyrene in equally strong solution. In our animals, we noted the appearance of carcinoma after ninety days of painting with benzpyrene, but we did not observe at that time a single carcinoma in mice treated with methylcholanthrene. However, papilloma seemed to develop more readily under the influence of methylcholanthrene than under that of benzpyrene.

The dissociation of cell migration from proliferation in the course of regeneration of the epidermis is not an effect of benzpyrene alone; it is also an effect of methylcholanthrene and perhaps of other carcinogenic agents. This does not imply, however, that it is necessarily correlated to the ability of these substances to produce cancer. As discussed previously, the increased keratinization occurring as a consequence of the intensified proliferation of the epithelium or of changes in the base of the wound may play a role in the disturbance of the motility of the cells. Experiments are in progress to analyze this phenomenon further.

## SUMMARY

Methylcholanthrene applied to the epidermis of mice before the making of a wound influences the course of regeneration in a way similar to benzpyrene. The application of methylcholanthrene for two weeks or for one month intensifies proliferation and migration of the epithelial cells and thus hastens the healing of the wound. But after three months of treatment with methylcholanthrene, cell migration is markedly inhibited and wound healing delayed in spite of the greatly increased proliferation of the epidermal cells. Between these two phases there is an intermediate stage in which the effect of methylcholanthrene differs from that of benzpyrene so far as the inhibition of cell migration is only transitory in the case of methylcholanthrene and is subsequently compensated for by rapid epithelization of the wound. The effect of methylcholanthrene on healing of wounds is thus a more quantitatively graded one than that of benzpyrene.

5. Nicod, J. L., and Regamey, J.: Bull. Assoc. franç. p. l'étude du cancer **27**:706, 1938.

# A PRESACRAL CYST APPARENTLY ARISING FROM THE NEURENTERIC CANAL IN A NEWBORN INFANT

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Anomalous developments and tumors not infrequently occur in the sacrococcygeal region. Tumors are relatively rare in the newborn. Tumor ventral to the sacrum was found by Calbet,[1] who collected 203 cases, to occur once in 34,582 births. Whittaker and Pemberton[2] stated that the incidence is 1 in every 40,000 registrations at the Mayo Clinic, reporting 22 cases.

This region passes through complicated phases of embryologic development. Hundling[3] stated that while the entoderm is forming the caudal intestine, the dorsal cord and the dorsal canal, the mesoderm the connective tissue, the blood vessels, the vertebrae and the muscles, and the ectoderm the primitive streak, the medullary tube and its vestiges, there is a continuation between the central canal of the spinal cord and the primitive alimentary canal around the caudal extremity of the notochord. This communication between the cord and the gut is known as the neurenteric canal. When the proctodeum, or primitive anus, invaginates to form part of the cloacal chamber, it meets the gut some distance anterior to and above the point where the neurenteric canal opens into it. Hence there is for a time a segment of intestine behind the anus termed the postanal gut. This, as well as the neurenteric canal, later becomes obliterated. The development of the urogenital tract is closely allied with these changes.

Guis and Stout[4] stated that the neurenteric canal is highly transitory in man and normally disappears before the neural groove is converted into a closed tube. Eternod[5] described a 2.1 mm. embryo in which the neurenteric canal was observed.

Middeldorpf[6] in 1885, described tumors occurring in this region, and the term Middeldorpf tumors has commonly been applied to them since then. Whittaker and Pemberton[2] expressed the opinion, however, that this term should more correctly be limited to the teratoid lesions which arise from the postanal gut.

According to Ewing,[7] the embryologic structures which give rise to these growths are chiefly the fovea coccygea and coccygeal vestiges of the neurenteric canal, the postanal gut, the neurenteric canal and the proctodeal membrane. The structure of many of these tumors suggests origin from various sources such as the coccygeal body, bone, cartilage and the neurenteric canal rather than from the postanal gut. Various descriptive terms have been applied to tumors occurring in this region both in children and in adults: "teratoma," "dermoid cyst," "Middeldorpf tumor," "meningeal cyst," "ependymal glioma," "neuroblastoma," "chondroma," "giant cell tumor," "myoma," "fibroma," "lipoma," "cloacal vestiges," "parasitic fetus," "sarcoma," "carcinoma," and "endothelioma." A large proportion are mainly cystic in character, while others are solid, with small cysts scattered throughout.

Teratoma arising in the coccygeal region and having cysts lined with neural elements has been described by Raven[8], Engelmann,[9] Scheuermann,[10] Pearse[11] and Law.[12] Hundling[3] reported 5 cases of presacral tumor of the adult; in these cases the tumor was composed of nerve tissue and was diagnosed as ependymal glioma. Guess[13] described a tumor that involved presacral and postsacral regions in an infant and diagnosed it as neurocarcinoma. Metastases were present in the inguinal nodes. He assumed that

From the Department of Pathology, Roosevelt Hospital.

1. Calbet, J.: Contribution a l'étude des tumeurs congenitales d'origine parasitaire de la régione sacro-coccygienne, Paris, G. Steinheil, 1893.
2. Whittaker, L. D., and Pemberton, J. deJ.: Ann. Surg. **107**:96, 1938.
3. Hundling, H. W.: Surg., Gynec. & Obst. **38**: 518, 1924.
4. Guis, J. A., and Stout, A. P.: Arch. Surg. **37**: 268, 1938.
5. Eternod, A. C. F.: Anat. Anz. **15**:181, 1898.
6. Middeldorpf, K.: Virchows Arch. f. path. Anat. **101**:37, 1885.
7. Ewing, J.: Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders Company, 1940, p. 1065.
8. Raven, R. W.: Brit. J. Surg. **23**:337, 1935.
9. Engelmann: Arch. f. klin. Chir. **72**: 942, 1903.
10. Scheuermann, H.: Arch. f. klin. Chir. **88**: 310, 1908-1909.
11. Pearse, H. E.: Surg., Gynec. & Obst. **33**:164, 1921.
12. Law, A. A.: Surg., Gynec. & Obst. **17**:340, 1913.
13. Guess, H. C.: Tr. Am. Proct. Soc. **37**:113, 1936.

it arose from the neurenteric canal. Mallory [14] reported a glioma situated in the postsacral region. Peyron [15] maintained that no genuine vestiges of cysts arising from the neurenteric canal had ever been described.

We wish to report a cyst lined with glia and ependymal cells which occurred in the caudal area of a newborn child.

A girl 3 days of age was admitted to the Roosevelt Hospital as a patient of Dr. James Thompson. A large cystic mass was present in the sacrococcygeal region. Prenatally, because of the large size of the mother's abdomen, it had been suspected that twins were present, but roentgen examination had revealed only one fetus and had given no evidence of the cyst. The latter had

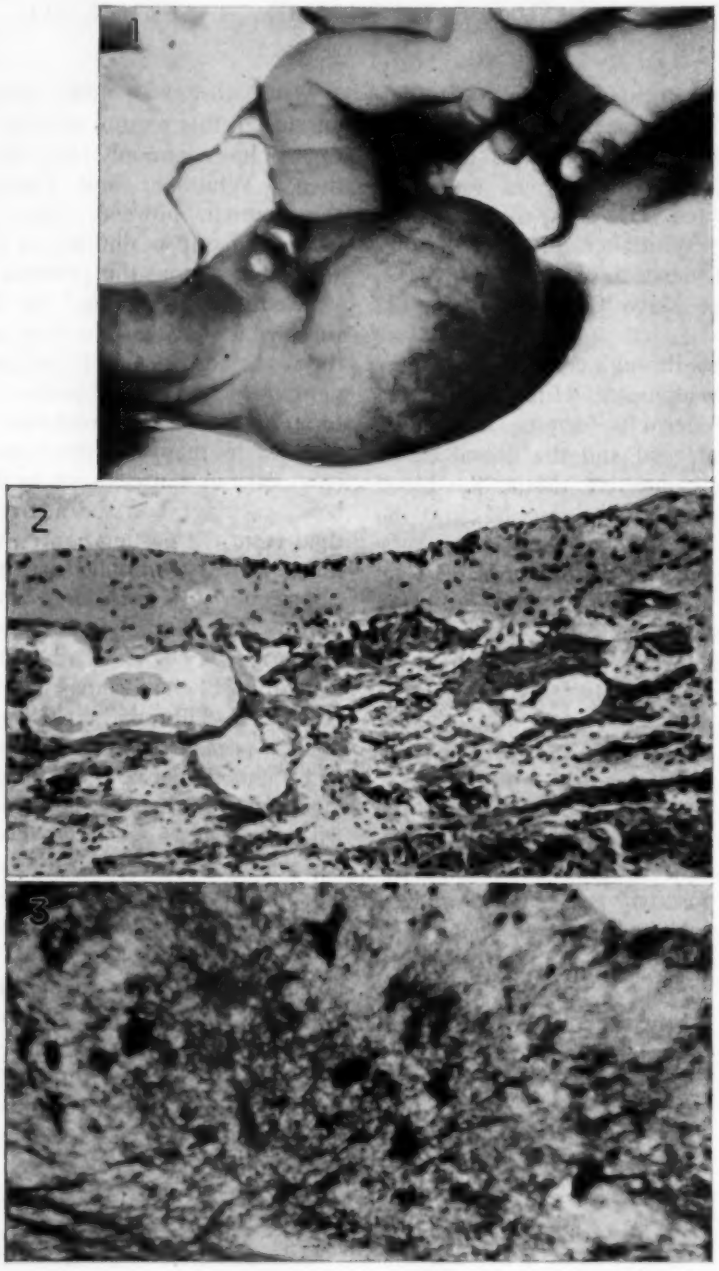


Fig. 1.—Anterior view of a cyst situated posterior to the anus.

Fig. 2.—Section from the wall of the cyst (× 150). Note the dark row of ependymal cells on the surface of the lining, resting on a narrow zone of glia. The glia extends for short distances into the stroma.

Fig. 3.—Cajal's silver stain demonstrating well formed astrocytes (× 600).

14. Mallory, F. B.: J. M. Research **13**:113, 1904-1905.

15. Peyron, A.: Bull. Assoc. franç. p. l'étude du cancer **17**:613, 1928.

not interfered with labor, although at the time of birth it was the size of a small grapefruit. Within the three days following birth it had increased to the size of the child's head. Urination and defecation were not interfered with (fig. 1).

The infant was well developed and normal except for the cyst. The skin was tightly stretched over the mass, which transilluminated well. It lay posterior to the rectum and extended into the pelvis, and the sacrum and the coccyx could be palpated on its posterior aspect. Compression of the cyst did not cause ballooning of the fontanels. Neurologic examination disclosed no abnormality.

Roentgen films showed a mass in the region of the perineum and the buttocks. The sacrum and the coccyx were underdeveloped, and the last two segments were displaced posteriorly. Along what appeared to be a septum were minute deposits of calcium.

Laboratory studies of the blood and the urine gave negative results.

At operation, the cyst was found to be rather firmly attached to the posterior aspect of the anus and distal part of the rectum and to the anterior aspect of the sacrum. It was removed intact, and the wound was closed primarily. Infection of the wound complicated the postoperative course, but the child was discharged on the forty-sixth day with complete healing.

Pathologic examination revealed a cystic mass measuring 10 by 12 cm. and weighing 340 Gm. Approximately half of its surface was covered with smooth thin skin. It transilluminated throughout. It contained slightly turbid watery fluid wth a faintly greenish tinge. The lining was everywhere smooth and gray.

The fluid contained 0.2 per cent protein, and its $p_H$ was 7.8; the amylase value was 51 units; the sugar content was 60 mg. per hundred cubic centimeters.

Microscopic sections revealed a thin covering of skin with short rete pegs and slight keratinization of the surface layers of the epidermis. The structure of the corium was normal. Sections cut from multiple areas showed a thin lining layer of glial tissue (fig. 2) with short extensions into the surrounding fibrous stroma. The glia was everywhere fully differentiated. Cajal's stain (fig. 3) showed well developed astrocytes and fibrillary stroma. In many areas cells perpendicularly arranged rested on this underlying glial tissue.

## COMMENT

The case is unique in that the cyst was lined throughout with well differentiated glial tissue, everywhere uniform in thickness. The fact that ependymal cells were present only in scattered areas can be explained on the basis of the rapid enlargement of the cyst following birth, which left only scattered remnants of the ependymal lining as the cyst wall was stretched. This rapid enlargement must have been due in part at least to the liberation of the infant with the cyst from the confined uterine space. Clinical examination produced no evidence of a connection with the central nervous system, and postoperatively no leakage of spinal fluid occurred.

The structure of the cyst wall and its position anterior to the sacrum indicate that it arose from the neurenteric canal. The main element of the wall was the neural tissue. The only mesodermal element present was the stroma supporting the blood vessels and the glial lining. In the areas examined, no muscle, bone, cartilage or epithelial elements were seen. It is therefore a simple cyst of neural origin and cannot be classified as a teratoma. Hansmann [16] expressed the opinion that frequently when a remnant of the neurenteric canal is the source of a tumor, there is an anterior sacral defect. In this case the sacrum was underdeveloped.

## SUMMARY

A presacral cyst with a lining composed of fully differentiated glial and ependymal tissue was surgically removed from a newborn infant. Its structure and position indicated origin from the neurenteric canal.

16. Hansmann, G. H.: Surg., Gynec. & Obst. **42**:124, 1926.

# CORONARY ARTERIOSCLEROSIS AND MYOCARDIAL INFARCTION AS STUDIED BY AN INJECTION TECHNIC

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A more accurate concept of the significance of sclerosis of the coronary arteries in relation to myocardial disease has been achieved in recent years. The development of such a concept has been aided by an injection and dissection technic devised by M. J. Schlesinger.[1]

Between July 14, 1943 and May 18, 1944, adult hearts from the autopsy service of the Mary Hitchcock Memorial Hospital, Hanover, N. H., were examined by this technic. After injection of the vessels and roentgen examination of the heart, the coronary arteries were dissected, and all occlusions and narrowings were recorded as to location and length of the portion of vessel involved. In narrowing without occlusion, the degree of constriction was estimated. Slight changes were designated as grade I and extreme changes as grade IV. Anastomoses were likewise recorded.

The myocardium was examined by splitting the left ventricle from apex to base parallel to the pericardium, thus exposing the largest possible surface of the myocardium in one plane. The septum was similarly split. Other incisions were made perpendicular to the first to reveal lesions lying in other planes. Recent infarcts and scars were described. From ten to twenty sections representing all regions of the heart were taken for microscopic study. The observations were correlated with the clinical findings.

## OCCLUSIONS OF THE CORONARY ARTERIES

Seventy hearts were examined. In 12 the coronary arteries were seen to be occluded. The distribution of the occlusions in age and sex groups is indicated in table 1.

TABLE 1.—*Distribution of Occlusions of the Coronary Arteries in Age and Sex Groups*

| Age Group | Males | No. with Occlusions | Females | No. with Occlusions | Total of Persons | No. with Occlusions |
|---|---|---|---|---|---|---|
| 20-29 | 1 | 0 | 1 | 0 | 2 | 0 |
| 30-39 | 2 | 0 | 2 | 0 | 4 | 0 |
| 40-49 | 4 | 0 | 4 | 0 | 8 | 0 |
| 50-59 | 9 | 1 | 6 | 1 | 15 | 2 |
| 60-69 | 11 | 2 | 8 | 1 | 19 | 3 |
| 70-79 | 10 | 3 | 7 | 2 | 17 | 5 |
| 80-89 | 3 | 1 | 1 | 1 | 4 | 2 |
| 90-99 | 0 | 0 | 1 | 0 | 1 | 0 |
| Total | 40 | 7 | 30 | 5 | 70 | 12 |

In the 12 hearts with occlusions, thirty-one points of obstruction were found. Eight of these were recent, and twenty-three were old. The recent obstructions were all produced by thrombosis with or without intimal hemorrhages and were all estimated as less than two weeks old. All remaining occlusions were designated as "old occlusions." Horn and Finkelstein [2] pointed out the difficulty of separating the lesions into those due to old acute occlusions and those due to gradual atherosclerotic narrowing.

TABLE 2.—*Distribution of Occlusions in the Three Systems of the Coronary Arteries*

| | Left Descending | | | Left Circumflex | | | Right | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| | Stem | Branch | Total | Stem | Branch | Total | Stem | Branch | Total | |
| Recent | 4 | 2 | 6 | 0 | 1 | 1 | 1 | 0 | 1 | 8 |
| Old | 3 | 8 | 11 | 1 | 4 | 5 | 4 | 3 | 7 | 23 |
| Total | 7 | 10 | 17 | 1 | 5 | 6 | 5 | 3 | 8 | 31 |

Multiple complete obstructions in single cases were seen. In 1 case there were one fresh and five old occlusions. In a second there were four recent and three old occlusions. The average length of the portion of vessel involved in old lesions was 0.3 cm. Seven involved portions measured 0.2 cm. or less. Thirteen points of obstruction were in the three main stems and eighteen in their branches. The system of vessels most often involved was the left descending coronary artery and its branches (table 2).

From the Department of Pathology, Dartmouth Medical School, and the Laboratories of the Mary Hitchcock Memorial Hospital, Hanover, N. H.

1. Schlesinger, M. J.: Am. Heart J. **15**:528, 1938.

2. Horn, H., and Finkelstein, L. E.: Am. Heart J. **19**:655, 1940.

### INTERARTERIAL ANASTOMOSIS

Three of 11 hearts with old occlusions showed no gross scars (table 3). Two of 5 hearts with recent occlusions of main stems showed no recent macroscopic infarcts (table 7). Occlusion without infarction can be explained only in the presence of a rich collateral circulation. Anastomoses were demonstrated in all hearts with severe coronary arteriosclerosis. Among the remaining hearts, collateral circulation was indicated only in several instances of marked cardiac hypertrophy.

### OLD OCCLUSIONS WITHOUT INFARCTS OR COMPLAINTS REFERABLE TO THE HEART

Three patients in the group of 12 with occlusions gave no history of complaints referable to the heart. In their myocardiums no areas of grossly visible scarring were seen (table 3). Coronary arteriosclerosis was severe; however, only 1 of the hearts was of markedly increased size, the heart with the least sclerosis of the coronary arteries. In only 1 case was a moderately high blood pressure recorded, the case in which the smallest heart was noted. Factors requiring an increased blood supply were generally slight, and there was no element apparent other than arteriosclerosis to limit the flow of blood through the coronary arteries.

### ANGINA PECTORIS

In the group of patients (table 4) in whom there was an extreme degree of coronary arteriosclerosis or in whom there was an increased demand for blood supply in the myocardium, the inadequacy of the coronary circulation was indicated clinically during increased activity by angina pectoris. This insufficiency of the coronary circulation was only relative and was relieved on resumption of rest. The data on 9 patients with angina pectoris are summarized in table 4. When sclerosis was slight, other factors decreasing coronary flow or calling for increased cardiac activity were found as abnormalities. In the smallest heart, two main trunks were completely occluded, and all three main stems showed extreme narrowing. In the eighth patient only one main stem was involved in marked narrowing, but long-standing extreme hypertension with prolonged cardiac decompensation was present. In the ninth patient there was only slight sclerosis of the coronary vessels. A stenosed aortic valve, however, might have served to decrease the coronary blood flow and, at the same time, to increase the demand on the myocardium.

TABLE 3.—*Data on Patients Who Had Old Occlusions Without Scars*

| Age of Patient | Sex | Heart Weight, Gm. | Estimated Maximum Normal Heart Weight,* Gm. | Blood Pressure | | Occlusions | Systems with Grade III or IV Narrowing |
|---|---|---|---|---|---|---|---|
| | | | | Systolic | Diastolic | | |
| 74 | M | 390 | 373 | 115 | 75 | 2 stems | 3 |
| 67 | F | 270 | 250 | 180 | 90 | 2 branches | 3 |
| 80 | F | 386 | 268 | 170 | 82 | 2 branches | 1 |

* According to H. L. Smith (Am. Heart J. 4 : 79, 1928).

TABLE 4.—*Data on Nine Patients with Angina Pectoris*

| Age of Patient | Sex | Heart Weight, Gm. | Estimated Maximum Normal Heart Weight, Gm. | Blood Pressure | | Old Occlusions | | Systems with Narrowing of Grade III or IV | Complicating Lesions | Macroscopic Scars |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Systolic | Diastolic | Stems | Branches | | | |
| 56 | F | 340 | 366 | 170 | 90 | 2 | 1 | 3 | ..... | + |
| 52 | M | 400 | 356 | 150 | 90 | 1 | 1 | 3 | ..... | + |
| 63 | M | 470 | 322 | 170 | 90 | 1 | 0 | 3 | ..... | + |
| 60 | M | 470 | 372 | 160 | 90 | 1 | 0 | 3 | ..... | + |
| 71 | M | 400 | 368 | 180 | 110 | 1 | 0 | 2 | ..... | + |
| 78 | M | 500 | 310 | 190 | 100 | 0 | 2 | 3 | ..... | + |
| 73 | M | 540 | 370 | 130 | 80* | 0 | 0 | 3 | ..... | + |
| 49 | F | 585 | 268 | 280 | 160 | 0 | 0 | 1 | ..... | + |
| 54 | M | 530 | 310 | 100 | 85 | 0 | 0 | 0 | Aortic stenosis | ? |

* Recorded in the presence of fibrillation and extreme cardiac decompensation.

### MYOCARDIAL INFARCTION

Myocardial anoxia, if severe and prolonged, may lead to infarction with typical symptoms and typical physical and laboratory findings. In table 5 are summarized data on 3 patients with acute occlusion followed by infarction. The occluding lesions in every instance proved to be thromboses with or without intimal hemorrhages. In all cases typical symptoms and signs of infarc-

tion were present. The infarcts were irregular in outline and poorly demarcated.

A striking feature in each instance was the extreme degree of arterial obstruction withstood by the heart before its circulation became so limited as to result in changes incompatible with life. Necrosis of a large area of heart muscle most commonly follows acute occlusion of a coronary artery, but similar changes are seen in the absence of recent total arterial obstruction. Summarized in table 6 are the data on a patient who had infarction without occlusion. Ten weeks before death he had severe substernal pain; the duration was approximately three and one-half hours; the onset occurred during exertion. No program of treatment was followed. There were later episodes of "stinging chest pain." The patient died of general peritonitis. A large

Table 5.—*Data on Patients with Recent Occlusion Followed by Infarction*

| Age of Patient | Sex | Heart Weight, Gm. | Estimated Maximum Normal Weight, Gm. | Duration of Illness | Duration of Pain | Estimated Age of Infarct | Old Occlusions | | Systems with Grades III or IV Sclerosis | Recent Occlusions | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Stems | Branches | | Stems | Branches |
| 54 | F | 340 | 366 | 4 days | Prolonged | 4 days | 2 | 1 | 3 | 2 | 2 |
| 78 | M | 500 | 310 | 13 days | Prolonged | 13 days± | 0 | 2 | 3 | 1 | 0 |
| 74 | F | 400 | 226 | 10 hours | 10 hours | Hours | 0 | 5 | 3 | 1 | 0 |

Table 6.—*Data on a Patient Who Had Infarction Without Occlusion*

| Age of Patient | Sex | Heart Weight, Gm. | Estimated Maximum Normal Weight, Gm. | Blood Pressure | | Systems with Grade III Sclerosis | Estimated Age of Infarct |
|---|---|---|---|---|---|---|---|
| | | | | Systolic | Diastolic | | |
| 51 | M | 575 | 371 | 150 | 100 | 2 | 2-3 mo. |

signs of infarcted heart muscle are encountered. In table 7 are the data on 2 patients who had thrombosis of the coronary arteries without corresponding infarcts though pain, and in 1 patient shock strongly suggested myocardial infarction. There was no friction rub, leukocytosis, fever or characteristic electrocardiographic picture of myocardial infarction. Acute occlusions were present, but no grossly recognized infarcts were detected though the estimated age of these occlusions was such that necrosis of heart muscle could have occurred.

Table 7.—*Data on Two Patients Who Had Recent Occlusion Without Recent Infarct*

| Age of Patient | Sex | Heart Weight, Gm. | Estimated Maximum Normal Heart Weight, Gm. | Blood Pressure | | Duration of Thrombus | Recent Occlusion (Location) | Old Occlusion | Systems with Grade III or IV Narrowing |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Systolic | Diastolic | | | | |
| 62 | M | 470 | 322 | 170 | 90 | Days | 1 stem | 0 | 3 |
| 53 | M | 400 | 356 | 150 | 90 | Days | 1 stem | 2 stems | 3 |

poorly demarcated myocardial infarct in the final stages of organization was observed. This case cannot be labeled one of angina pectoris, as the pain was not relieved at rest but was prolonged. It was not "coronary occlusion," as no occlusion existed. Schlesinger and co-workers [3] have used the term "coronary failure" to describe such a condition, while Master and his colleagues [4] have preferred the diagnosis "acute coronary insufficiency."

This case supports the thesis that the diagnosis "acute coronary occlusion" can be made logically only when characteristic symptoms are initiated under conditions in which there has not been a call for increased supply of blood to the heart or in which other factors which might rapidly decrease the oxygen carried to the myocardium, such as shock and hemorrhage, are absent. Furthermore, acute occlusion of the coronary arteries may occur without myocardial infarction. Therefore, it is unwise to assume that infarction has occurred unless the telltale

That large scars seen in the myocardium are almost universally the aftermath of infarction is generally accepted. The cause of small scattered scars is less evident. Brown [5] concluded that ischemia was the cause of most of the scarring in a group of 110 hearts with "myocardial fibrosis."

Grossly visible scarring was observed in 26 of the 70 hearts in the present series. The scars ranged from those covering several square centimeters to those consisting of a few scattered areas

3. Blumgart, H. L.; Schlesinger, M. J., and Zoll, P. M.: J. A. M. A. **116**:91, 1941.

4. Master, A. M.; Jaffe, H. L.; Dack, S., and Gershman, A.: (*a*) J. Mt. Sinai Hosp. **8**:820, 1942; (*b*) Am. Heart J. **27**:803, 1944.

5. Brown, M. R.: Am. J. M. Sc. **184**:707, 1932.

of gray streaking. Table 8 indicates the distribution of the lesions as to age and sex, and table 9 summarizes the anatomic or clinical abnormalities considered possibly responsible for the lesions.

TABLE 8.—*Distribution of Scars in Sex and Age Groups*

| | 20-39 | 40-49 | 50-59 | 60-69 | 70-79 | 80-89 | 90-99 | Total |
|---|---|---|---|---|---|---|---|---|
| Males..... | 0 | 0 | 5 | 7 | 6 | 1 | 0 | 19 |
| Females..... | 0 | 1 | 1 | 0 | 4 | 0 | 1 | 7 |

TABLE 9.—*Possible Etiologic Factors in the Production of Myocardial Scars*

| Etiologic Factors | Cases |
|---|---|
| Coronary arteriosclerosis with hypertension..... | 12 |
| Coronary arteriosclerosis without hypertension..... | 4 |
| Hypertension alone ..... | 4 |
| Aortic stenosis ..... | 3 |
| Episodes of severe hemorrhage from chronic duodenal ulcer ..... | 1 |
| Hodgkin's disease ..... | 2 |

There were 16 hypertrophied hearts in this group; the enlargement of 13 was associated with hypertension and that of 3 with aortic stenosis. All hearts listed as showing arteriosclerosis contained at least one main stem with sclerosis interpreted as grade III or IV. Abnormalities were present in each heart of a type to either increase its need for blood or to decrease the oxygen carried to it by the blood. Two cases of Hodgkin's disease were apparent exceptions. Ten hearts of the group showed no significant arteriosclerosis.

In table 10 are summarized the data on patients whose myocardium showed either microscopic zones of necrosis with acute inflammatory exudate or small areas of granulation tissue. These lesions were histologically identical with larger obvious infarcts, differing from them only in size. Such lesions in similar circumstances have been described by others.[6] They were interpreted as minute infarcts, and it is believed that their end stage would appear similar to the focal myocardial scars seen in all but the sixth case. In this group as in that in table 9 a variety of conditions were present to produce a discrepancy between the heart's need and the supply of oxygen.

A review of this study shows that among conditions accompanying myocardial scarring or infarction the most frequent is sclerosis of the coronary arteries. However, often other abnormalities appeared simultaneously with or independently of arteriosclerosis. It is thus indicated that coronary arteriosclerosis is only one of several factors which may act to produce myocardial anoxia.

## COMMENT

Though many abnormalities frequently accompany scarring or infarction of heart muscle, clinicians and pathologists alike tend to consider only coronary arteriosclerosis. Yet there is abundant evidence[7] that collateral circulation may develop rapidly and effectively to assume the function of the constricted vessels. The importance of this collateral flow is paramount in determining the effect of occlusive disease of the coronary arteries on the myocardium.

As sclerosis of coronary arteries progresses and new anastomotic channels develop, the path of blood flow may become quite complex, so that areas once supplied by one artery may now be supplied in part or entirely by a different artery whose origin may be far removed from the first. Further narrowing or sudden occlusion in this circumstance could be expected to render a different intensity and pattern of ischemia than

TABLE 10.—*Data on Patients in Whom Focal Lesions of the Myocardium Had Occurred*

| Age of Patient | Sex | Heart Weight, Gm. | Estimated Maximum Normal Heart Weight, Gm. | Old Occlusions | Recent Occlusions | Systems with Grade III or IV Narrowing | Healed Macroscopic Scars | Other Important Factors |
|---|---|---|---|---|---|---|---|---|
| 79 | M | 555 | 383 | 0 | 0 | 0 | + | Calcareous aortic valvular disease |
| 50 | M | 1,040 | 412 | 0 | 0 | 0 | + | Calcareous aortic valvular disease |
| 74 | F | 400 | 226 | Branches, 5 | 0 | 3 | + | Hypertension |
| 63 | M | 470 | 322 | 0 | Stems, 1 | 3 | + | Hypertension |
| 52 | M | 400 | 356 | Stems, 1; branches, 1 | Stems, 1 | 3 | + | Hypertension |
| 71 | F | 296 | 193 | 0 | 0* | 0 | 0 | Organizing pneumonia; pulmonary fibrosis |

6. (*a*) Gross, H., and Spark, C.: Am. Heart J. **14**:160, 1937. (*b*) Fahr, G.: J. A. M. A. **105**:1396, 1935.

7. (*a*) Lowe, T. E.: Am. Heart J. **21**:326, 1941. (*b*) Spalteholz, W.: Die Arterien der Herzwand, Leipzig, S. Hirzel, 1924. (*c*) Gross, L.: The Blood Supply to the Heart, New York, Paul B. Hoeber, 1921. (*d*) Blumgart, H. L.; Schlesinger, M. J., and Davis, D.: Am. Heart J. **19**:1, 1940. (*e*) Prinzmetal, M.; Kayland, A.; Margoles, C., and Tragerman, L. J.: J. Mt. Sinai Hosp. **8**:933, 1942. (*f*) Wiggers, C. J.: Am. Heart J. **11**:641, 1936. (*g*) Blumgart, H. L.; Gilligan, D. R.; Zoll, P. M.; Freedberg, A. S., and Schlesinger, M. J.: Tr. A. Am. Physicians **57**:152, 1942. (*h*) Eckstein, R. W.; Gregg, D. E., and Pritchard, W. H.: Am. J. Physiol. **132**:351, 1941. (*i*) Schlesinger.[1]

similar changes in a normal set of vessels. These differences are demonstrated in experimental and autopsy studies. Ligation of a coronary artery in a dog's heart produces a well circumscribed area of infarction.[8] Similarly it has been observed that recent large circumscribed infarcts in human hearts are usually the results of sudden occlusion in a set of relatively lightly involved vessels.[9] In contrast patchy necrosis or "infarction at a distance" [10] may occur in sudden arterial occlusion in hearts with severe coronary arteriosclerosis. At other times recent myocardial changes may be absent.[11] Lowe has shown by injecting dye into branches of sclerosed coronary vessels of human hearts that the distribution of stained myocardium is patchy. Many have noted patchy distribution of scarring in hearts with severe arteriosclerosis.[12]

Variations in the fundamental pattern of coronary arteries may further affect the result of occlusive disease. Several authors [13] have observed a reciprocal relationship in the distribution of the right and the left circumflex coronary arteries. Schlesinger [13a] divided hearts into three groups on this basis. He demonstrated that large anastomoses develop with varying facility according to the type of circulation present. Therefore, a given amount of sclerosis could be expected to produce varying amounts of damage according to the pattern of vessels present.

The ability of the coronary circulation to adapt itself readily to occlusive arterial disease accounts for the many observations that extreme arteriosclerosis to the point of occlusion may be found in hearts of persons who have never complained of angina pectoris or of symptoms of cardiac decompensation and in whose hearts no areas of scarring or evidences of failure are found. In such persons the coronary circulation supplies the heart muscle with sufficient oxygen to maintain it at rest and during the usual activity of the individual. This and other studies [14] indicate that this group of people generally lack conditions other than coronary arteriosclerosis which might produce myocardial anoxia.

Such additional conditions indicated as important by clinical, pathologic and experimental studies can be divided into three groups: (1) increased oxygen requirement of the heart; (2) decreased oxygen-carrying function of the blood; (3) decreased volume flow of the coronary circulation.

The first condition may be produced by factors augmenting the heart's activity, such as hypertension,[15] thyrotoxicosis,[16] valvular heart disease [17] and physical exertion.[18] Hypertrophy of a heart requires an increased blood supply, and, according to some workers, is important in decreasing the vascular bed in relation to the muscle mass.[19] Also it may produce ischemia of the individual fiber by increasing the size of the fiber and thus the distance that oxygen must diffuse.[20] This thesis, however, is contested.[21]

A decrease in the oxygen-carrying function of the blood may occur in anemia,[22] carbon monoxide poisoning,[23] exposure to oxygen-poor atmospheres [24] and in certain pulmonary diseases.[25]

A decrease in volume of coronary circulation may occur in such conditions as occlusion of coronary ostiums in syphilis,[26] rheumatic arteritis,[27] periarteritis nodosa,[28] myocardial arteriolar

8. Karsner, H. T., and Dwyer, J. E.: J. M. Research **34**:21, 1916.

9. Lowe.[7a] Blumgart and others.[7d]

10. Bean, W. B.: Ann. Int. Med. **12**:71, 1938. Blumgart and others.[7d]

11. Saphir, O.; Priest, W. S.; Hamburger, W. W., and Katz, L. N.: Am. Heart J. **10**:567 and 762, 1935. Eckstein and others.[7h]

12. Friedberg, C. K., and Horn, H.: J. A. M. A. **112**:1675, 1939. Master and others.[4a] Blumgart and others.[7d]

13. (*a*) Schlesinger, M. J.: Arch. Path. **30**:403, 1940. (*b*) Whitten, M. B.: Arch. Int. Med. **45**:381, 1930. (*c*) Gross.[7c]

14. Blumgart and others (footnotes 3 and 7 *d*).

15. Levine, V.: Arch. Path. **18**:331, 1934. Fahr.[6b]

16. Menne, F. R.; Jones, O. N., and Jones, N. W.: Arch. Path. **17**:333, 1934.

17. Blumgart and others.[3] Friedberg and Horn.[12]

18. (*a*) Wood, F. C., and Wolferth, C. C.: Arch. Int. Med. **47**:339, 1931. (*b*) Riseman, J. E. F., and Brown, M. G.: Am. Heart J. **18**:150, 1939.

19. Wearn, J. T.: Bull. New York Acad. Med. **17**: 754, 1941. Gross and Spark.[6a]

20. Harrison, T. R.; Ashman, R., and Larson, R. M.: Arch. Int. Med. **49**:151, 1932. Gross.[6a] Wearn.[19]

21. Dock, W.: J. Exper. Med. **74**:177, 1941.

22. Büchner, F., and von Lucedow, W.: Beitr. z. path. Anat. u. z. allg. Path. **93**:169, 1934. Master, A. M., and Jaffe, H. L.: J. Mt. Sinai Hosp. **7**:26, 1940. Friedberg and Horn.[12]

23. Christ, C.: Beitr. z. path. Anat. u. z. allg. Path. **94**:111, 1934.

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sclerosis,[29] aortic valvular disease,[30] cardiac decompensation [31] and certain arrhythmias.[32] Any condition provoking prolonged episodes of low blood pressure may be associated with infarction of heart muscle.[33] A derangement of reflex or hormonal control of coronary blood flow must be a significant element.[34] Experiments in animals [35] and studies of the human heart [31] lend abundant evidence to support this conclusion. For example, demonstrations of the effect of cold in angina pectoris are striking.[36] These factors may well account for discrepancies in symptoms or in myocardial changes in cases with similar anatomic pictures.

Observations from the study of this series and data collected from the literature indicate that coronary arteriosclerosis, though occurring most frequently, is only one of many possible abnormalities that may act to produce an insufficient supply of oxygen in the myocardium.

The pattern of the vessels and the changes resulting in the coronary circulation from narrowing determine the site and, to varying degrees, the intensity of the ischemia. The more pronounced other factors become, the less pronounced must be the occlusive effect of coronary arteriosclerosis in the production of anoxia with the resulting symptoms and anatomic changes.

## SUMMARY

An unselected series of 70 adult hearts were studied by the Schlesinger injection technic.

Occlusions of the coronary arteries were demonstrated in 12 of the 70 hearts. In these 12 hearts thirty-one points of obstruction were demonstrated. Thirteen were in the main stems of the three principal coronary arteries. Eighteen were in the large branches.

Interarterial anastomoses were demonstrated in all hearts with pronounced arteriosclerotic narrowing. Only in the presence of marked hypertrophy were such anastomoses demonstrated in other hearts.

In 3 of 11 hearts with old occlusions of the coronary arteries there were no old infarcts. In 2 of 5 hearts with recent occlusions of the coronary arteries there were no corresponding recent infarcts.

In 1 of 4 hearts with recent infarcts there was no recent occlusion.

Grossly recognizable scars were present in the myocardium of 26 of the 70 hearts.

Data from this work and from the literature emphasize that coronary arteriosclerosis is only one of the many factors which may be responsible for the anatomic changes and the symptoms resulting from myocardial anoxia.

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# Case Reports

## PERIARTERITIS NODOSA PRODUCING ANEURYSM OF THE RENAL ARTERY AND HYPERTENSION

PETER A. HERBUT, M.D., AND ALISON H. PRICE, M.D., PHILADELPHIA

That one form of hypertension in man is of renal origin has been accepted by most observers. From a review of the literature it is apparent that the recorded cases may be divided into two groups, namely, those in which the primary disease affected the main renal artery or its orifice thereby producing narrowing of the lumen and renal ischemia, and those in which the disease affected primarily the renal parenchyma itself. The reported causes of the former include arteriosclerosis,[1] periarteritis nodosa,[2] congenitally misplaced plug of smooth muscle,[3] dissecting aneurysm [4] and thrombosis of the main renal artery,[5] while the reported cases of unilateral disease of the renal parenchyma include pyelonephritis, renal calculi, hydronephrosis, infarcts, tuberculosis, cortical abscesses and hypernephroma.[2a]

In spite of the ever increasing number of reports on aneurysm of the renal artery [6] the relationship between such a lesion and renal hypertension has not been well established. In a recent review of the literature on obstructive lesions of the main renal artery, Yuile [1d] found that hypertension was seen in only 8 of 77 reported cases of aneurysm of this vessel and concluded that "available data provide little more than a suggestion of an occasional association between this type of lesion and hypertension." Because of the infrequency with which an etiologic relationship has been found to exist between aneurysm of the renal artery and hypertension, the role which periarteritis nodosa might play in such a combination must indeed be small. One can be certain that this disease is not one of the common causes of aneurysm of the main renal artery or of any of its immediate branches, for these causes, as listed by Mathe,[6a] Howard, Suby and Harberson [6b] and Lowsley and Cannon,[6c] consist of trauma, congenital defects in the arterial wall, arteriosclerosis, syphilis and severe infections such as pneumonia, pleurisy, rheumatic fever, endocarditis, malaria, dysentery and chronic nephritis. Although in each of the three reviews just referred to periarteritis nodosa is also listed as a possible cause, in none is the number of recorded cases stated nor is any reference given to a report of cases in which it was a cause. Thus Mathe [6a] said that "only 9 of the 56 cases reviewed had arteriosclerosis, only 2 were suffering from syphilis and an equally small number presented periarteritis nodosa." Howard, Suby and Harberson [6b] said the same thing in practically the same words, and Lowsley and Cannon [6c] reported that "periarteritis nodosa although occasionally generalized displays a particular affinity for the renal artery and a small number of cases have shown this pathologic entity at autopsy."

Thus, because aneurysm of the main renal artery or its immediate branches developing on the basis of a previous periarteritis nodosa is not common, and because the association of such an aneurysm with the development of hypertension has never been satisfactorily demonstrated, we are recording 2 cases.

### REPORT OF CASES

CASE 1.—A 44 year old Italian man, who had no noteworthy previous illnesses, consulted his physician because of gross hematuria and dull pain in the left costovertebral angle in July 1943. At the time of this and other visits to his physician his blood pressure ranged from 116 to 130 mm. of mercury systolic and 68 to 74 mm. diastolic. Because his symptoms were unaccounted for, he was sent to another hospital for study, where both an indigo carmine test and a retrograde pyelogram showed no abnormality in either kidney. A urinalysis disclosed albumin (a trace), erythrocytes and a few pus and epithelial cells. There were 17,600 leukocytes per cubic millimeter of blood with 80 per cent polymorphonuclear cells and 3 per cent eosinophils. The hematuria and the lumbar pain disappeared, and he was entirely well until December 1943, when severe headaches, dizziness and blurring of vision developed. With these symptoms he was admitted to the Jefferson Medical College Hospital on April 1, 1944.

Physical examination gave negative results except for a blood pressure of 170 mm. of mercury systolic and 120 mm. diastolic and "diffuse hypertensive retinal changes with arteriosclerosis, grade III." The eyegrounds showed both arteriolar changes in the form of perivasculitis, calcification and obliteration and, of more recent origin, edema of the nerve heads, cotton wool exudates and flame-shaped hemorrhages. Several

From the Department of Clinical Laboratories of Jefferson Medical College Hospital and the Department of Medicine of Jefferson Medical College.

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urinalyses disclosed a specific gravity around 1.008, traces of albumin in some specimens and a few hyaline casts and leukocytes but no erythrocytes. A phenolsulfonphthalein test showed less than 5 per cent of the dye excreted in fifteen minutes and 10 per cent in thirty minutes. The urea clearance was 70 per cent of normal. The Wassermann and Kahn tests of the blood and the Wassermann test of the spinal fluid were negative, and the blood urea nitrogen and proteins were normal. An intravenous urogram and a retrograde pyelogram disclosed that the calices and the pelvis of each kidney were normal.

Two weeks after admission the patient suddenly became dizzy, flaccid paralysis of the left side of his body developed and he died in coma twenty-four hours later. Prior to death the blood pressure was 230 mm. of mercury systolic and 110 mm. diastolic.

*Necropsy* (eight and a half hours after death).—The left kidney weighed 80 Gm. and measured 9 by 6 by 2 cm. Its upper third was irregularly atrophied to about one-half its normal size (fig. 1). The external

Fig 1 (case 1).—Photograph of the left kidney showing ischemic atrophy of its upper portion and a saccular aneurysm of the main branch of the renal artery supplying this portion of the kidney. The vessel and the aneurysm were opened. Both are shown retouched in the photograph.

surface was superficially and deeply scarred, and to it the capsule was densely adherent. When the latter was stripped, the surface was in addition finely granular. The lower two thirds of the left kidney was normal in shape and size. The capsule stripped easily, leaving a smooth, moist, glistening, reddish brown surface. Cut surfaces showed in the upper portion a pale brown parenchyma with a marked reduction of both the cortex and the medulla and an obscuring of the demarcations between the two. There was likewise no sharp line of delineation between the atrophic upper third of the kidney and the relatively normal lower two thirds. Cut surfaces of the lower portion of the left kidney revealed essentially normal cortex and medulla with a sharp corticomedullary demarcation. The left renal artery along with its aortic orifice and its branch to the lower portion of the kidney were normal. The main branch to the upper portion of the kidney, however, contained, 1 cm. distal to its origin, a saccular aneurysm 1.5 cm. in diameter. Externally the latter was well circumscribed and was attached to the artery posteriorly by a pedicle measuring about 0.9 cm. in diameter. The wall of the aneurysm was fibrotic and was 0.2 to 0.3 cm. thick. The inner surface was smooth, and the lumen contained a recent soft reddish gray blood clot. The ostium between the aneurysm and the renal artery was oval and measured 0.5 cm. in its greatest diameter. The inner surface of the aneurysm was uninterruptedly continuous with that of the vessel. The lumen of the latter at its junction with the ostium of the aneurysm was considerably narrowed, measuring 0.2 cm. across. That just distal to the aneurysm was so small that it was found only with difficulty. It measured less than 0.1 cm. in diameter. Beyond this constriction the renal vessel expanded again to an internal circumference of approximately 0.6 cm. and pursued its normal course into the renal parenchyma.

The right kidney weighed 210 Gm. and measured 11 by 8 by 4 cm. The capsule stripped easily, leaving a smooth, reddish brown surface. Cut surfaces showed sharp corticomedullary demarcations and no abnormalities. The larger vessels were not prominent. The entire extrarenal portion of the right renal artery, including its aortic orifice, was normal.

Along the attachment of the mesentery to the small intestine there were many shotlike, round, firm nodules measuring from 0.1 to 0.5 cm. in diameter. Sometimes they were partly within the wall of the intestine but usually they were within the mesentery itself, and most of them were attached to the terminal ramifications of the mesenteric artery. Cut surfaces varied from gray to brown and were for the most part solid. Except for scattered atherosclerotic plaques in the aorta and the coronary arteries, there were no other grossly detectable vascular abnormalities.

The heart weighed 400 Gm. The left ventricle measured 1.5 cm. and the right 0.5 cm. in thickness. There was some thickening of the papillary muscles and trabeculae of the left ventricle but no other abnormality. The lungs disclosed congestion, edema and areas of consolidation in their posterior portions.

The brain weighed 1,340 Gm. The hemispheres were symmetric, but the gyri were flat and the sulci were partially obliterated. The vessels at the base of the brain were tortuous and showed atheromatous plaques. The right frontal lobe just anterior to the head of the caudate nucleus contained a recent hemorrhage, 2.5 by 4 cm. in diameter, which had extended into and completely filled the right ventricle and had pushed the ventricular system to the left. The left lateral ventricle and the third ventricle also contained blood. Throughout the midbrain and the pons there were small hemorrhages, but nowhere were aneurysms seen.

Microscopic sections of many of the circumscribed mesenteric nodules that grossly appeared to be partly within the wall of the intestine revealed fibrosed aneurysmal dilatations of the terminal ramifications of the mesenteric artery. They were located in the serosa or between the longitudinal and circular muscle layers and at the periphery often showed either longitudinal or cross sections of the vessels from which they sprang (fig. 2*A*). The portions of the arteries directly opposite the aneurysms were as a rule normal in all respects. As the ostium of the aneurysm was approached, however, there was increasing fibrosis of the adventitia, gradual disappearance of the external elastic lamina, fibrous replacement of the media, gradual or sudden disappearance of the internal elastic lamina and a varied degree of proliferation of the subendothelial connective tissue of the intima. Sometimes this was so great as to obliterate the lumen of the ostium. Beyond this the artery was completely lost in a thick concentric mass

of dense fibrous tissue in which the coats of the vessel were no longer recognized. The lumens of the aneurysms were usually small, comprising less than one half of the entire external diameter. They were either empty or filled with well preserved erythrocytes or recently clotted blood. Throughout the areas of fibrosis there were deposits of golden brown pigment which took a positive stain for iron. These deposits were particularly abundant in the adventitia, where they were both free and within phagocytes. Occasionally the periphery also contained a few plasma cells and lymphocytes.

The upper branch of the left renal artery was cut into several blocks so as to include the junction of the artery with the aneurysm, the aneurysmal wall itself and the artery just distal to the aneurysm. The portion of the vessel directly opposite the aneurysm was essentially normal. As the latter was approached, however, the following changes were noted: increasing fibrosis of the adventitia; some attenuation of the external elastic lamina; increasing replacement of the media with fibrous tissue; in some areas preservation and in others

In all sections, throughout the areas of fibrosis but particularly prominent in the adventitia there were deposits of golden brown pigment which gave a positive reaction for iron. In these same areas there were varied numbers of plasma cells, lymphocytes and phagocytes. Calcification was not seen. There was no recognizable old or recent thrombus either within the aneurysm or within the renal artery itself.

Microscopic sections of the ischemic portion of the left kidney showed a condensation of all the elements. In the cortex there were a few scattered wedge-shaped areas of complete fibrosis, where the glomeruli were represented as round, completely hyalinized globules. At the apexes of these triangular areas, which pointed to the medulla, there were larger arteries that were partially or completely occluded by extensive subintimal proliferation of fibrous tissue without hyalinization. In some of these vessels the media was also partially or completely replaced with fibrous tissue. In all of them the adventitia and surrounding connective tissue showed marked fibrosis with a peripheral sprinkling of lympho-

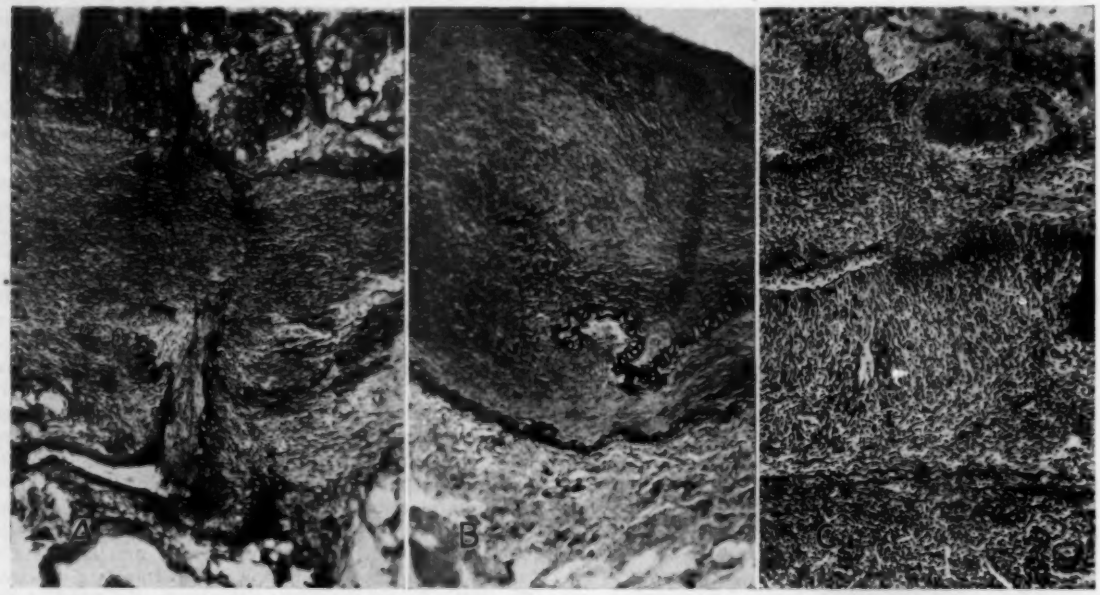


Fig. 2.—*A* (case 1), photomicrograph of a terminal mesenteric artery as it enters an aneurysm, showing increasing adventitial and medial fibrosis, loss of the external elastic lamina, splitting and finally disappearance of the internal elastic lamina, marked subendothelial proliferation of the intima, and fluid and recently clotted blood in the lumen. Elastic tissue stain; × 48.

*B* (case 1), photomicrograph of the aneurysm of the left renal artery at its junction with the vessel, showing fibrosis of the adventitia, some attenuation of the external elastic lamina, increasing replacement of the media with fibrous tissue, fibrillation and complete disappearance of the internal elastic lamina and severe fibrosis of the intima. Elastic tissue stain; × 48.

*C* (case 2), photomicrograph of a section of a large artery in the hilus of the right kidney, showing diffuse infiltration with cells of inflammatory origin, granulation tissue replacement of the adventitia, almost complete destruction of the internal elastic lamina, marked thickening of the intima by early granulation tissue and a recent thrombus in the lumen. Hematoxylin and eosin; × 72.

fibrillation, attenuation or complete disappearance of the internal elastic lamina; increasing proliferation of the subendothelial connective tissue of the intima (fig. 2 *B*). These changes became increasingly severe until the fibrosed adventitia was separated from the completely fibrosed and fused media and intima by varied amounts of the external elastic lamina. This constituted the wall of the aneurysm itself. The changes in the renal artery at its exit from the aneurysm were similar to those just described with the exception that the shape of the vessel was still maintained and that subendothelial proliferation of the intima with marked encroachment on the lumen was even more conspicuous.

cytes and plasma cells. Throughout the rest of this portion of the kidney, where the tissue was only condensed, the arteries showed little or no fibrosis and the arterioles showed no evidence of endarteritis obliterans. The glomeruli were normal. The tubular epithelium showed some degeneration. The interstitial connective tissue was condensed but not otherwise increased and showed no inflammation.

Histologic sections of the grossly normal portion of the left kidney and of the right kidney were similar. There were only a few scattered fibrosed glomeruli; the rest were entirely normal. The tubular epithelium showed considerable degeneration, and the interstitial

connective tissue was not increased. The larger and the smaller arteries were remarkably free of fibrosis or arteriosclerosis. Whereas some of the arterioles were normal, many showed marked endarteritis obliterans, in some instances to such a degree that the lumen was almost entirely obliterated.

Microscopic sections of the brain from the edge of the ventricular hemorrhage showed degeneration of the brain substance and scattered perivascular hemorrhages. The cerebral arteries and arterioles about 1 cm. from the area of destruction showed considerable intimal and adventitial fibrosis. The periphery of the latter contained plasma cells, lymphocytes, occasional polymorphonuclear leukocytes and scattered deposits of golden brown pigment.

Sections of the rest of the organs together with portions of the pectoralis major muscle and the diaphragm failed to disclose recent or old periarteritis nodosa or other contributory changes.

Case 2.—A Negro man 30 years old was admitted to the Jefferson Medical College Hospital with a history of pains in the legs and the lumbar region of six days' duration and generalized pain, weakness and anorexia of four days' duration. Physical examination disclosed injection of the pharynx, enlargement of the cervical lymph nodes and extreme tenderness of the calf muscles. The temperature was 100 F.; the blood pressure was 180 mm. of mercury systolic and 130 mm. diastolic; the blood count was normal except for a leukocyte count of 16,000 cells per cubic millimeter, and the Wassermann and Kahn tests of the blood were negative.

During the next two weeks the pains persisted; the temperature varied from 100 to 102 F., and the blood pressure from 180 to 140 mm. of mercury systolic and 130 to 100 mm. diastolic. On the fifteenth day after admission the patient began to have localized pain, tenderness and muscle guarding in the right upper quadrant of the abdomen. Four days later this pain became severe; the leukocytes numbered 26,000 per cubic millimeter, and the blood pressure was 110 mm. of mercury systolic and 80 mm. diastolic. Within the next four days his condition rapidly deteriorated, and he died in shock twenty-three days after admission.

*Necropsy.*—An extensive recent retroperitoneal hemorrhage on the right side extended from the upper pole of the right kidney to the pelvic brim. This originated in a tear of the parenchyma of the lower pole and lateral margin of the right kidney that measured 2.5 cm. in length and 2.0 cm. in depth. The rupture was filled with recently coagulated blood, and the surfaces were irregular, dark red and partially necrotic. In the depth of the defect there was a ruptured aneurysm, 1.5 cm. in diameter, which also contained recently clotted blood. When the capsule and perirenal hemorrhage were stripped away, the right kidney weighed 370 Gm. and measured 15 by 8 by 6 cm. The left kidney weighed 420 Gm. and measured 16 by 8 by 5 cm. Both capsules stripped easily, leaving smooth external surfaces. Cut surfaces of each kidney showed numerous raised, brown to deep red nodules measuring 0.1 to 1.0 cm. across. They were scattered throughout the kidneys but were particularly prominent at the hili, where they were seen to be direct expansions of the main branches of the renal arteries as they entered the renal substance. The nodules were firm and on section disclosed central areas of fluid and clotted blood. The cortices and medullas were broad and the demarcations between them sharp. The extrarenal portions of the renal arteries and their aortic orifices were grossly normal.

Similar deep red nodules, often seen to be grossly related to vascular ramifications, were encountered in the omentum, the mesentery, the intestines, the pancreas, the gallbladder, the liver and the pericardium. In the intestines they were found in the lower part of the ileum and in the colon and in all instances were intramural. There was considerable enlargement of the abdominal lymph nodes. The heart weighed 520 Gm., and the lungs showed congestion and edema. Permission to examine the brain was not granted.

Microscopically the medium-sized and smaller arteries of the kidneys, intestines, mesentery, omentum, lymph nodes, gallbladder, liver, adrenal glands, pancreas and heart were affected with fulminating acute periarteritis nodosa. The vessels of the lungs and the spleen were not involved. In each kidney, in addition to sections made from the cortex, the smaller extrarenal branches of the renal arteries and their continuations into the hilus of the kidney were also examined and showed either patchy or complete involvement in the inflammatory process (fig. 2 *C*).

All the lesions were in the acute stage, showing no attempt at healing. The adventitia and a varying portion of the immediately surrounding tissue were densely infiltrated by polymorphonuclear leukocytes, eosinophils, plasma cells and lymphocytes. In some of the arteries, where the process was less severe, there were fewer inflammatory cells and increasing degrees of fibroblastic proliferation. The media was variously involved. In some arteries only the outer or the inner portion or both showed fibrinoid necrosis. In others the entire musculature was replaced with deep pink–staining fibrinoid material and contained inflammatory cells and nuclear fragments. In those vessels in which only the outer portion of the media and the adventitia were involved, the internal elastic lamina was often entirely normal. When, however, the inner portion of the media and the intima were involved, the internal elastic lamina was either fibrillated or, in the more severely involved arteries, completely absent. Usually the intima was necrotic and infiltrated by cells similar to those found in the adventitia, but occasionally there was considerable fibroblastic proliferation of the subendothelial connective tissue. The endothelium in the involved areas had disappeared. The lumens of the vessels were empty or filled with necrotic material and clotted blood. While many of the vessels still maintained their normal contours, some showed small and others large aneurysmal dilatations. Often the aneurysms were so large that the wall was composed of a thin rim of inflammatory tissue in which vascular structures could no longer be recognized.

In addition to the vascular changes the kidneys showed varied degrees of infiltration of the interstitial connective tissue with plasma cells, lymphocytes and eosinophils. These infiltrations appeared to follow the distribution of the vessels. Except for small focal areas of necrosis about some of the aneurysms the glomeruli and the tubules were normal. Many of the afferent arterioles were also normal, but others showed advanced sclerosis with hyaline replacement of the intima. Endarteritis obliterans, however, was not found.

## COMMENT

In the first case the abdominal pain that occurred ten months before death was due to periarteritis nodosa of the left renal artery supplemented, perhaps, by similar involvement of the branches of the superior mesenteric artery. It was undoubtedly further aggravated when the lumen of the renal artery was occluded to such a degree that the whole upper portion of the left kidney became ischemic. That this ischemia was accompanied with true wedge-shaped infarcts

of the cortex is borne out by the areas of cortical fibrosis observed at necropsy. Either the ischemia or the infarcts could have been responsible for the hematuria. As hypertension did not develop until sometime after the episode of hematuria, it was undoubtedly produced by the renal ischemia. Death from cerebral hemorrhage is one inevitable result of hypertension even in those cases in which the cerebral vessels are not particularly weakened by a previous disease. In this case the presence in some of the cerebral arteries of intimal and adventitial fibrosis together with peripheral plasma cells, lymphocytes, occasional polymorphonuclear leukocytes and deposits of golden brown pigment is evidence that these vessels were involved in the same inflammatory process as were the branches of the superior mesenteric and left renal arteries. Whether this was enough to weaken the wall, and so predispose to a rupture, or whether a small, undiscovered aneurysm was the source of the cerebral hemorrhage remains a matter of conjecture.

Pathologically, there is little doubt that the renal aneurysm in this case was on the basis of previous periarteritis nodosa. Grossly this is supported by other evidence of periarteritis nodosa in the form of numerous aneurysms of the branches of the superior mesenteric artery—a site of predilection for this disease. Histologically, too, sections of the renal vessel and aneurysm were similar to those of the mesenteric vessels and each corresponded to the healed stage of periarteritis nodosa described by Arkin.[7] In each the involved portion of the vessel showed fibrous tissue replacement of the adventitia, the media and especially of the subendothelial connective tissue of the intima, with marked or complete occlusion of the lumen. The internal elastic lamina was partially or completely destroyed. Scattered deposits of iron pigment, especially in the adventitia, spoke of old hemorrhages, and the occasional presence of peripheral plasma cells and lymphocytes indicated the passing phase of the granulation tissue stage.

That the second case was one of acute periarteritis nodosa needs no further elaboration or proof. It differs from the former in that the course was fulminating, the extrarenal portions of the renal arteries were not affected, and there was no grossly demonstrable renal ischemia. Many of the main branches of both renal arteries within the hili of the kidneys, however, were affected by the process, resulting in the formation of scattered aneurysms. Similar dilatations were found in the medullas and cortices. In either or both of these locations the blood supply to the kidneys could have been impeded sufficiently to liberate a "toxic substance" responsible for the hypertension. The absence of grossly visible atrophic changes in the kidneys does not in any way detract from such an explanation, for the arterial constriction may not have been of sufficient severity to produce such changes. In dogs Goldblatt[8] has demonstrated that the kidneys "may show but little change or may undergo atrophy, depending upon the severity of the constriction." Then, too, the entire course of the illness may have been too fulminating for atrophic changes to develop. The time element, however, plays no part in the development of hypertension, for in dogs[8] and in a few human patients, such as one described by Yuile,[1d] hypertension developed immediately after the renal artery was occluded.

Finally, the reports on arteriolar changes in the ischemic and the nonischemic kidney are far from uniform. In dogs[8] the arterioles in the kidneys show no changes whereas those in other organs do. In man, Blackman[1a] and Richardson[1b] observed arteriosclerosis and arteriolonecrosis in both ischemic and nonischemic kidneys. Leadbetter and Burkland[3] and Stewart,[1c] on the other hand, reported an absence of arteriolar changes in the affected kidney, while Yuile[1d] described not only an absence of arteriolosclerosis in the ischemic kidney but also marked sclerosis of the arterioles of the uninvolved kidney. In our first case the ischemic portion of the left kidney showed no arteriolar changes while the nonischemic portion of the same kidney together with the right kidney showed advanced endarteritis obliterans but no sclerosis. In the second case, although most of the vessels showed no changes, advanced sclerosis was seen in a few scattered arterioles in both kidneys. Endarteritis obliterans, however, was not seen. In neither case was there arteriolonecrosis.

## SUMMARY

A review of recent literature discloses that cases of hypertension of renal origin may be divided into (1) those in which renal ischemia is produced by diseases affecting primarily the renal arteries and (2) those in which the renal parenchyma itself is primarily involved.

In a case of periarteritis nodosa of the renal arteries there was an old periarteritic renal aneurysm which produced narrowing of the vascular lumen, with renal ischemia, hypertension and death from cerebral hemorrhage following. In a second case multiple acute periarteritic aneurysms of the intrarenal branches of both renal arteries produced hypertension, with death resulting from rupture of one of the aneurysms.

The sequence of events in the first case indicates that an aneurysm of an extrinsic portion of a renal artery can produce hypertension provided the lumen of the vessel is occluded to a degree great enough to produce renal ischemia.

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8. Goldblatt, H.: Experimental Hypertension Induced by Renal Ischemia, in Harvey Lectures, 1937-1938, Baltimore, Williams & Wilkins Company, 1938, p. 237.

# HISTOLOGIC DIAGNOSIS OF RABIES

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Among 75 subjects suspected of having rabies, (68 animals and 7 human beings) 52 were found actually to have the disease. A comparative study was made of the different methods of morphologic diagnosis, such as the examination for Negri bodies in Ammon's horn or the histologic examination of the ganglion nodosum of the vagus nerve, the superior cervical sympathetic ganglion, or the mesencephalon and the medulla. The method which I [1] found independently and published in 1941 had been described in 1900 by van Gehuchten and Nélis but had been completely forgotten after the discovery of Negri bodies. In almost 50 per cent of our 52 cases of rabies we could not find Negri bodies in Ammon's horn by Lentz's method, although the simultaneous examination of the ganglion nodosum in the same cases always disclosed the alterations described in the foregoing paragraph.

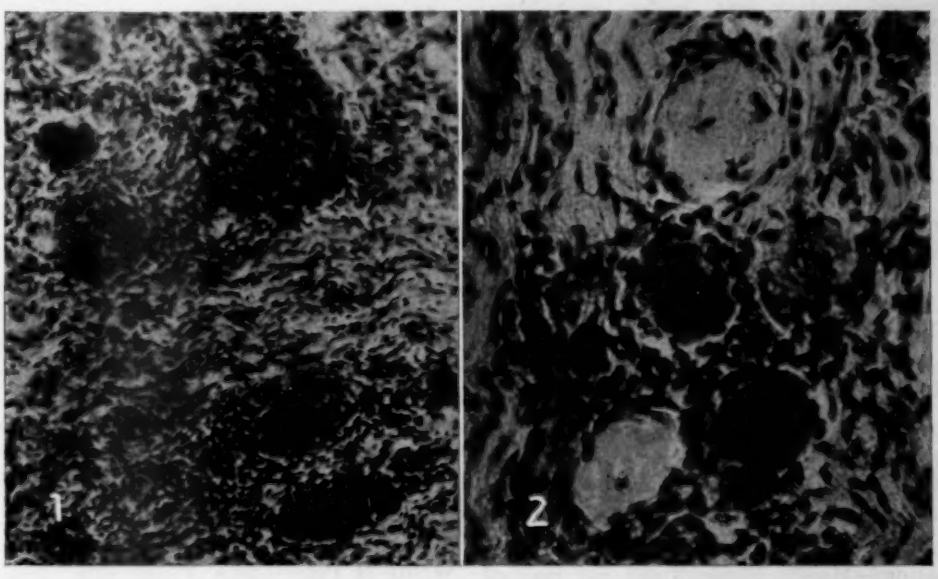


Fig. 1.—Diffuse rabic ganglionitis in a dog. Note two van Gehuchten nodules (residual nodules of Nageotte), with disappearance of the corresponding nerve cells, proliferation of capsular cells and inflammatory cell infiltration (neuronophagia). Nissl's method with cresyl violet; × 180.

Fig. 2.—Ganglion nodosum of a dog with rabies. Note two nerve cells with chromatolysis marked by pallor and nuclear rests. Nearby is an inflammatory infiltration. Nissl's method with cresyl violet; × 310.

The histologic examination of the ganglion nodosum always shows in cases of rabies characteristic degenerative alterations of the nerve cells, with or without inflammatory pericellular infiltrations, in focal or diffuse form, with marked neuronophagic proliferation of the capsular cells (satellites)—alterations that were constant in all of the 52 cases concerned here. Even though they are not specific they are not found in this form in the other diseases that must be considered in the differential diagnosis of rabies.

The method consists of a rapid staining of frozen sections from different planes of the ganglion nodosum of each side with cresyl violet, and it permits the making of a diagnosis in less than one hour after the arrival of a suspected animal at the laboratory. If the brain is not destroyed or putrefied, a control examination of Ammon's horn, embedded in paraffin and stained by Lentz's method, a procedure which is more dependable than the taking of a smear, is added, but this delays the diagnosis eight to ten hours.

From the Institute of Pathological Anatomy, University of Concepción, Concepción, Chile; director, Prof. Dr. Ernesto Herzog.

This is a summary of an article in Spanish to appear in Revista sudamericana de morfologia 1945, vol. 3.

1. Herzog, E.: Bol. Soc. de biol. Concepción, **15**:101, 1941; Arch. chilena morfol. **4**:299, 1942.

A positive result of the examination of the ganglion nodosum is conclusive even if the Negri bodies are absent or cannot be sought. If the examination of the ganglion nodosum and that of Ammon's horn give negative results, histologic examination of the mesencephalon and the medulla in frozen sections may be added if the brain is in good condition, the examiner looking for the characteristic alterations of rabies encephalitis. If the results of two or three morphologic tests are negative, it is not worth while to inoculate animals, as the bitten person may be considered free from rabies and spared the disagreeable antirabic injections. In doubtful cases inoculation of white mice may always be done, which gives a definite result in fifteen days.

The investigation of the ganglion nodosum has also the advantage that this structure is easily accessible even in beheaded animals. Furthermore, it is more resistant to putrefaction. In this way the examinations give a result in one or two days, even in many instances in which the result of the examination for Negri bodies is negative.

## Notes and News

**Appointments.**—At the school of medicine of the University of Pittsburgh, Elwyn L. Heller has been appointed assistant professor of pathology and parasitology in the place of Andrew Wallhauser, who has resigned. Dr. Heller is also pathologist to the Presbyterian Hospital of Pittsburgh.

Gilbert Dalldorf, director of laboratories of Grasslands Hospital, Valhalla, N. Y., has been appointed director of laboratories and research of the New York State Department of Health, succeeding A. B. Wadsworth, retired.

Bjarne Pearson, associate professor of pathology in Tulane University, New Orleans, has been appointed professor of pathology in the University of Vermont.

William O. Russell, formerly assistant professor of pathology in Washington University, St. Louis, is now pathologist to the Santa Barbara Cottage Hospital, visiting pathologist to the Santa Barbara General Hospital and to the St. Francis Hospital, Santa Barbara, and assistant professor of pathology in the University of Southern California, Los Angeles.

**Resignation.**—Marcos Fernan-Nunez has resigned as professor and director of the department of pathology and bacteriology in the school of medicine of Marquette University, Milwaukee.

**Unveiling of Portrait.**—The continuous service of Francis Carter Wood, director of the pathologic laboratories and of radiotherapy, as a member of the staff of St. Luke's Hospital, New York, for fifty years was marked by the unveiling on February 14 of his portrait, painted at the direction of the board of managers of the hospital.

**Death.**—John C. Grill, professor and director of the department of pathology and bacteriology in the school of medicine of Marquette University since December 1944, died on March 17 at the age of 52 years.

## Books Received

MICROBIOLOGY AND PATHOLOGY. By Charles F. Carter, B.S., M.D., instructor in microbiology and pathology, Parkland Hospital School of Nursing, and director of Carter's Clinical Laboratory, Dallas, Texas. Third edition. Pp. 777, with 225 illustrations. Price $3.50. St. Louis: C. V. Mosby Company, 1944.

THE MARIHUANA PROBLEM IN THE CITY OF NEW YORK. Sociological, Medical, Psychological and Pharmacological Studies. By the Mayor's Committee on Marihuana. Pp. 220. Price $2.50. Lancaster, Pa.: Jaques Cattell Press, 1944.

APPROVED LABORATORY TECHNIC: CLINICAL PATHOLOGICAL, BACTERIOLOGICAL, MYCOLOGICAL, VIROLOGICAL, PARASITOLOGICAL, SEROLOGICAL, BIOCHEMICAL AND HISTOLOGICAL. John A. Kolmer, M.S., M.D., Dr.P.H., Sc.D., LL.D., L.H.D., F.A.C.P., professor of medicine in the school of medicine and the school of dentistry, Temple University; director of the Research Institute of Cutaneous Medicine, Philadelphia, and Fred Boerner, V.M.D., associate professor of clinical bacteriology, Graduate School of Medicine, and assistant professor of bacteriology, School of Medicine, University of Pennsylvania; bacteriologist, Graduate Hospital of the University of Pennsylvania, Philadelphia. Fourth edition. Price $10. Pp. 1,017, with 346 illustrations. New York and London: D. Appleton-Century, 1945.

THE MARCH OF MEDICINE. The New York Academy of Medicine Lectures to the Laity, 1944. Price $1.75. Pp. 121. New York: Columbia University Press, 1945.

LABORATORY MANUAL FOR ELEMENTARY PHYSIOLOGY. By Lalia V. Walling, assistant professor, and Kenneth Siler, formerly instructor, University of Kansas. Fourth edition. Price $1.50. Pp. 187, illustrated. St. Louis: The C. V. Mosby Company, 1945.

THE NATIONAL FOUNDATION FOR INFANTILE PARALYSIS, INCORPORATED. ANNUAL REPORT, 1944. Pp. 52. New York.

NATIONAL RESEARCH COUNCIL. ORGANIZATION AND MEMBERS, 1944-1945. Pp. 88. Washington, D. C.: National Research Council, 1944.